THE REGULATION OF LUTEINIZING HORMONE SECRETION BY GONADAL STEROIDS IN THE HEN (*GALLUS DOMESTICUS*)

by

Susan Caroline Wilson

B.Sc.(Hons) University of Hull

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DECLARATION

I declare that this thesis has been composed by myself and that it consists of the results of my own work.

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SUMMARY

The measurement of luteinizing hormone (LH) by radioimmunoassay in plasma samples taken at frequent intervals revealed an episodic pattern of secretion in cockerels and gonadectomized fowl with mean plasma LH concentration related to the frequency of episodic discharges. A similar pattern of secretory episodes could not be demonstrated in laying hens.

The lower concentration of LH in the plasma of laying hens is probably the result of the negative feedback effect of gonadal steroids. In the ovariectomized hen oestrogen exerted a stronger depressive effect on LH secretion than did progesterone, though sensitivity to oestrogen was reduced after exposure to multiple injections. There was evidence of a synergistic effect between oestrogen and progesterone in lowering LH secretion.

A single 2- to 4-fold rise in plasma LH levels occurred at 4 to 8 h before ovulation. Pre-ovulatory plasma LH levels started to rise only during the hours of darkness or shortly thereafter (lights on 07:00 h to 21:00 h). A smaller rise in LH concentration occurred at the onset of darkness and,in a few cases, also at the time of oviposition.

The injection of oestrone or oestradiol-17β at various stages of the ovulatory cycle did not stimulate LH secretion. Neither did oestradiol benzoate when injected into ovariectomized hens which were either untreated, or primed with oestrogen, progesterone, or a combination of both steroids.

An intramuscular injection of 0.05 to 2.0 mg progesterone/kg in ovariectomized hens primed with a combination of multiple oestrogen

and progesterone injections, induced an LH surge. The magnitude of the positive feedback response was dependent on the ratio of progesterone to oestrogen injected during priming treatment and, where oestrogen treatment was curtailed, the LH response to an injection of progesterone 72 h later was abolished. It therefore appears that oestrogen is necessary for maintaining the positive feedback response to progesterone. A facilitative role for oestrogen was also indicated by the observation that passive immunization with antiserum to oestradiol-17β on 2 successive days both delayed and reduced the pre-ovulatory LH surge. In contrast, the injection of antiserum to oestradiol-17β on a single day did not affect the pre-ovulatory LH surge 12 to 15 h later, suggesting that oestrogen is not directly involved in inducing the pre-ovulatory LH surge.

Injections of progesterone stimulated LH secretion at any time during the ovulatory cycle apart from the 4 h preceding an ovulation when LH levels were falling after the occurrence of a pre-ovulatory LH surge. Compared with the uniform LH response observed between 4 h after one ovulation and 12 h before the next, the response to progesterone was reduced during the 4 h before the start of the natural pre-ovulatory LH rise and enhanced when LH levels were rising.

Injections of 0.5 to 2.0 mg testosterone/kg caused an LH surge comparable in magnitude with the natural pre-ovulatory surge when given 22 to 26 h after the final ovulation of a sequence; at this time the ovary contains a mature follicle. In contrast, little or no response resulted from injections at 0 to 9 h after ovulation when the largest ovarian follicle would be less mature, or in the oestrogen-progesterone primed ovariectomized hen. It is likely that testosterone injections

at 22 to 26 h after ovulation stimulated the release of ovarian progesterone, which then induced an LH surge. Passive immunization with an antiserum raised against testosterone generally did not affect the pre-ovulatory LH surge, but disrupted the process of ovulation.

A positive feedback response to progesterone was first observed at 8 to 12 weeks before the onset of lay, when the comb, ovary and oviduct had started to grow and basal LH levels were beginning to rise. Rising LH levels were accompanied by an increased LH response to progesterone, although the LH response to synthetic LH-releasing hormone (LH-RH) remained constant. Three to four weeks before the first egg was laid, basal plasma LH levels started to fall, associated with a reduction in the LH response to LH-RH and progesterone. The final stage of sexual maturation occurred during the week before the onset of lay and though there was a further fall in sensitivity of the pituitary to synthetic LH-RH, injections of progesterone resulted in a prolonged release of LH. Multiple priming injections of progesterone at a time when basal LH levels were falling enhanced the positive feedback response which suggests that the final priming of the system is a result of increased progesterone secretion from the large maturing follicles.

Apart from being directly responsible for inducing LH release, progesterone also appears to be involved in the timing of oviposition. When injected at 0 to 6 h after an ovulation, the oviposition of the newly ovulated egg was delayed and occurred at a constant interval after injection. Also the succeeding ovulation was delayed in such a manner that the events of oviposition and ovulation simulate those occurring at the end and beginning of a normal sequence. It is suggested that progesterone may regulate the length of egg sequences.

INTRODUCTION

INTRODUCTION

Since the early part of the present century the domestic hen has been increasingly exploited on a commercial basis by the food industry and for this reason there has been considerable interest in methods of improving its reproductive efficiency. Particular attention has been given to increasing egg production by selective breeding, though further improvements may necessitate the development of new selection criteria based on a fundamental understanding of the ovulatory process. An investigation of the mechanism by which gonadal steroids regulate the secretion of the "ovulation-inducing" hormone, or as is widely accepted, luteinizing hormone (LH), may reveal endocrine factors involved in determining reproductive efficiency, and may be of value to the future development of laying strains of fowl.

The role of gonadal steroids in the regulation of LH release in the domestic fowl has been mainly deduced from measurements of the steroid production during the ovulatory cycle and on the occurrence of natural and steroid-induced ovulation. A sensitive and specific method for measuring LH in the blood of the fowl was unavailable until an homologous radioimmunoassay for fowl LH was developed recently by Follett, Scanes & Cunningham (1972).

However, the regulation of LH secretion has been extensively studied in mammals for several years and an understanding of the principles involved is a useful prelude to a study of LH secretion in the fowl.

I. REGULATION OF LH SECRETION IN MAMMALS

There is evidence of three types of feedback mechanisms involved in the regulation of gonadotrophin secretion in mammals. Gonadotrophin releasing factor may regulate its own secretion by means of an "ultra-short feedback" loop (Kawakami & Sakuma, 1974) or gonadotrophins may act at the hypothalamus to regulate the secretion of gonadotrophin releasing factor by a "short feedback" loop (Motta, Fraschini & Martini, 1969). The third, the regulation of gonadotrophin secretion by gonadal steroids, termed the "long feedback" loop, has been the mechanism most thoroughly investigated.

The concept of a reciprocal relationship between the gonadal steroids and gonadotrophins was first proposed by Moore & Price (1932). Early experiments had revealed the occurrence of gonadal atrophy following hypophysectomy. Similar effects were observed after prolonged oestrogen administration to female rats, and were attributed to a negative feedback action of oestrogen reducing gonadotrophin secretion. The fine control with which secretions of gonadal steroids and gonadotrophins regulate each other has been demonstrated in experiments whereby removal of one ovary caused compensatory growth of the remaining ovary, presumably by reducing gonadal steroid secretion, and thereby lifting its inhibitory action on gonadotrophin release (Donovan, 1967).

It is accepted that gonadotrophin secretion from the pituitary regulates the development of the ovary and that the secretion of gonadotrophins can itself be regulated by gonadal steroids, so

forming a feedback loop. Injections of gonadal steroids can exert both inhibitory and stimulatory effects on LH secretion, depending on circumstances such as dose of steroid, age of recipient animal or composition and concentration of steroids in the blood at the time of injection. In the female, there are two categories of LH secretion, "basal" or "cyclic", which are regulated by negative and positive "long feedback" loops respectively.

A. REGULATION OF BASAL LH SECRETION

LH secretion is considered to be "basal" at all times other than during the pre-ovulatory surge and there is considerable evidence that basal secretion is regulated by the negative feedback effects of ovarian steroids on the hypothalamo-hypophyseal complex. Thus, if the ovary is removed there is a marked increase in the concentration of LH in the blood, as for example in the adult rhesus monkey (Atkinson, Bhattacharya, Monroe, Dierschke & Knobil, 1970), rat (Gay & Midgley, 1969; Yamamoto, Diebel & Bogdanove, 1970), hamster (Goldman & Porter, 1970) and ewe (Reeves, O'Donnell & Denorscia, 1972; Diekman & Malven, 1973). Similarly, both pituitary and circulating LH levels were found to be higher in post-menopausal women with regressed ovaries and lower concentrations of steroids in the circulation than in pre-menopausal women (Franchimont, 1971).

In several mammals basal LH secretion is maintained by pulsatile discharges from the pituitary. This is particularly marked in intact males and in gonadectomized mammals of both sexes. The administration of oestrogen, and in some cases progesterone, depresses the elevated rate of LH secretion in ovariectomized mammals and

abolishes the pulsatile manner of LH discharge (see page 138).

It appears that when the negative "long feedback" loop has developed in the immature animal the hypothalamo-hypophyseal complex is more sensitive to oestrogen than after sexual maturation (Ramirez & McCann, 1963; Smith & Davidson, 1968; Eldridge, McPherson & Mahesh, 1974). These observations support the hypothesis that the onset of puberty is due to a reduction in the sensitivity of the hypothalamo-hypophyseal complex to the negative feedback effects of sex steroids which leads to a rise in gonadotrophin secretion. This, in turn, stimulates ovarian growth and the secretion of larger quantities of gonadal steroids until a final equilibrium is reached between the secretions of LH and gonadal steroids at puberty (Hohlweg, 1936; Byrnes & Meyer, 1951; Donovan & van der Werff ten Bosch, 1959; Ramirez & McCann, 1963). This event is associated with the onset of ovulatory cycles.

B. REGULATION OF CYCLIC LH SECRETION

Throughout most of the ovulatory cycle LH secretion is maintained at a low level by the negative feedback effects of gonadal steroids. However, in breeding females the basal pattern of LH secretion is interrupted at regular intervals by a steep pre-ovulatory increase in concentration of LH in the circulation.

In all mammalian species so far investigated, the LH peak at oestrous is preceded or accompanied by a surge in the circulating concentration of oestrogen. Ayalon & Shemesh (1974) reported, also, that a small progesterone peak occurred before the pre-ovulatory LH surge in the cow. However, all other recent studies suggest that

progesterone levels never start to rise until after the LH surge has been initiated. Species showing this sequence of events include humans (Mishell, Nakamura, Crosignani, Stone, Kharma, Nagata & Thorneycroft, 1971), the rhesus monkey (Weick, Dierschke, Karsch, Butler, Hotchkiss & Knobil, 1973), the chimpanzee (Reyes, Winter, Faiman & Hobson, 1975), the baboon (Stevens, Sparks & Powell, 1970), the cow (Wetteman, Hafs, Edgerton & Swanson, 1972; Lemon, Pelletier, Saumande & Signoret, 1975), the sow (Henricks, Guthrie & Handlin, 1972) and bitch (Jones, Boyns, Cameron, Bell, Christie & Parkes, 1973; Nett, Akbar, Phemister, Holst, Reichert & Niswender, 1975). The involvement of oestrogen in initiating the pre-ovulatory LH surge has been demonstrated by showing that injection of antisera to oestrogen will block the LH surge (Ferin, Tempone, Zimmering & Vande Wiele, 1969; Neill, Freeman & Tillson, 1971).

There are two main hypotheses to account for the way in which gonadal steroids regulate the pre-ovulatory release of LH. The most popular is that as ovarian follicles mature, they secrete increasing amounts of oestrogen, which, on reaching a critical blood concentration, exert a positive feedback effect at the hypothalamus. This stimulates the secretion of luteinizing hormone-releasing hormone (LH-RH), which in turn stimulates the pituitary to release LH (Barraclough, 1973). LH is believed to trigger the series of events leading to the process of ovulation. A second, and less popular hypothesis is that, since in the rat and woman oestrogen levels in the blood are normally falling when pre-ovulatory LH levels are rising (Vande Wiele, Bogumil, Dyrenfurth, Ferin, Jewelewicz, Warren, Rizkallah & Mikhail, 1970;

Gorski, Mennin & Kubo, 1975), it is a reduction in the negative feedback effects of high levels of oestrogen rather than a rise in oestrogen concentration which stimulates the release of LH (Mayuzumi, Ebihara & Matsumoto, 1971; Yen & Tsai, 1972). Supporting evidence for this hypothesis comes from the observation of Franchimont (1971) who found that when post-menopausal women are treated with oestrogen, a surge in LH secretion never occurs until the treatment is terminated. However, the observation in the hamster that oestrogen injected on the morning of pre-oestrous does not delay ovulation fails to support this hypothesis (Greenwald, 1965).

C. FEEDBACK SITES FOR GONADAL STEROIDS

Experiments involving lesioning or implanting gonadal steroids within selected areas of the brain have revealed the sites of positive and negative feedback.

1. Positive feedback sites

In the rat, electrical stimulation at brain sites extending from the medial pre-optic area, through the anterior hypothalamus to the median eminence caused a rise in plasma LH concentration and ovulation (Cramer & Barraclough, 1971; Kalra, Ajika, Krulich, Fawcett, Quijada & McCann, 1971). However, the anterior pituitary has been shown to possess the characteristics of a steroid target organ since it can take up and retain oestradiol (Kato & Villee, 1967; Notides, 1970) and Davidson, Weick, Smith & Dominguez (1970) reported a rise in gonadotrophin secretion following short-term implantation of oestradiol in the pituitary. In vivo, responses to LH-RH in the ewe and rat can be increased by pre-treatment with oestrogen (Arimura & Schally, 1971;

Reeves, Arimura & Schally, 1971a).

Experiments described by Ferin et al., (1969) and van Rees (1972) suggest that progesterone is not essential for spontaneous ovulation, although there is evidence of a synergistic effect between oestrogen and progesterone. Van Rees (1972) has suggested that progesterone could act at the mediobasal hypothalamus to sensitize this region to the stimulatory effects of oestrogen.

2. Negative feedback sites

In rats long-term implants of oestrogen in the basal tuberal region of the hypothalamus inhibit the rise in basal LH secretion after ovariectomy (Ramirez, Abrams & McCann, 1964). The negative feedback effect of oestradiol appears to involve stimulation of the synthesis of RNA which inhibits the secretory activity of the LH-RH neurons and hence blocks the release of LH-RH (McCann, 1974).

A single injection of oestrogen has a biphasic effect on pituitary sensitivity to LH-RH: initially sensitivity is diminished and subsequently is enhanced (Cooper, Fawcett & McCann, 1974; Libertun, Orias & McCann, 1974).

In vivo, pituitary responses to LH-RH can be decreased by progesterone in the cyclic rat (Martin, Tyrey, Everett & Fellows, 1974a) and anoestrous (Pant & Ward, 1973) and pro-oestrous (Hooley, Baxter, Chamley, Cumming, Jonas & Findlay, 1974) ewe.

II. THE GONADS AND REGULATION OF THEIR FUNCTION IN THE DOMESTIC HEN

Although information concerning the role of gonadal steroids in regulating LH secretion in the hen is scarce, extensive studies have been carried out to investigate patterns of ovulation and to determine the effects of gonadal steroids on the induction of ovulation.

A. THE GROWTH AND STRUCTURE OF THE OVARY

The ovary of the mature hen consists of an inner medulla and outer cortex, containing the developing follicles. Most of these are less than 1 mm diameter, and grow at an extremely slow rate lasting from several months to years (Marza & Marza, 1935). Of the follicles in which yolk deposition has occurred, several are uniform in size (about 5 or 6 mm diameter) and contain white yolk. When the follicles reach 9 to 10 mm diameter, yellow yolk begins to be deposited, and the follicles then develop rapidly to produce the hierarchy of about 5 or 6 graded follicles typically seen in the laying hen. During the 7 days preceding ovulation of a particular follicle, the ovum increases 16-fold in weight as the result of yolk deposition which is greatest between 9 and 3 days before ovulation (Warren & Conrad, 1939).

The involvement of gonadotrophins in maintaining ovarian activity was demonstrated by Hill & Parkes (1935) and Opel & Nalbandov (1961) who observed that after hypophysectomy there was a regression of the ovary.

Breneman (1955) studied the relationship between growth of the ovary, comb and pituitary during pullet development. He noted an

increase in the rate of comb growth from 16 to 18 weeks of age, associated with an acceleration of ovarian growth. Since comb growth is androgen-dependent, these findings suggested an increase in the ovarian secretion of androgens. However, studies involving radioimmunoassay have shown an increased secretion of oestrogen during development (Peterson & Webster, 1974; Senior, 1974) which reaches a maximum 3 weeks before the first egg is laid. An increase in the number and activity of pituitary basophils, presumed to secrete gonadotrophins, was found to occur at the time of increased rate of ovarian growth (Breneman, 1955).

B. THE PATTERN OF OVULATION AND OVIPOSITION

The first oviposition in pullets of most commercial strains of laying fowl occurs about 18 to 24 weeks after hatching. In regularly laying hens generally only one follicle a day ovulates and this is always the largest in the hierarchy of follicles of graded sizes seen in the ovary of an adult. At ovulation the stigma, which is relatively avascular and extends over half the circumference of the follicle, ruptures to release the yolk from the follicle (Phillips & Warren, 1937; Kraus, 1947). The ovum is then engulfed by the infundibulum and during the next 4 to 5 h passes through the magnum and isthmus to reach the uterus (shell gland) in which it spends a further 18 to 20 h. Oviposition occurs at least 24 h following ovulation (Warren & Scott, 1935).

Warren & Scott (1935) found that except at the beginning and end of a sequence the timing of oviposition and ovulation of the following ovum are generally closely related with ovulation succeeding

oviposition by 14 to 75 minutes in most cases.

The domestic hen lays eggs in sequences and except in the middle of very long sequences, each oviposition occurs later in the day than the previous one. This difference in the time of day between consecutive ovipositions is termed the "lag" (Fraps, 1954, 1955a), which is generally positive and is greatest between the first and second and between the penultimate and last eggs in a sequence. Lag is shortest in mid-sequence; in the middle of long sequences it may be occasionally negative. The first egg of a sequence is laid soon after the lights come on and the last between 8 and 10 h later in the day under a standard lighting schedule of 14 h light and 10 h darkness. The last egg terminates the sequence and is followed by a single day termed a "pause day" on which no oviposition occurs. On the following day an egg is laid soon after the lights come on to start another sequence.

Laying is confined to the hours of daylight in birds held on a standard lighting schedule, and if the photoschedule is reversed the period during which laying occurs is also reversed (Warren & Scott, 1936). When birds are kept in continuous light, ovipositions are distributed randomly throughout the 24 h day (Warren & Scott, 1936; McNally, 1947; Morris, 1961) though, in such conditions, noise or feeding regime (Fraps, Neher & Rothchild, 1947; McNally, 1947; Wilson Woodard & Abplanalp, 1964) or fluctuations in environmental temperature (Payne, Lincoln & Charles, 1965) will synchronize laying. Egg production can be increased by maintaining hens on a daylength greater than 24 h (Foster, 1968) with maximum sequence length achieved using a 26 h day (Byerley & Moore, 1941); this is the approximate

period elapsing between successive ovipositions in a sequence.

C. THE CONTROL OF OVULATION

1. Hormonal involvement

Direct evidence for a hormonal control of ovulation came from the studies of Fraps and co-workers who prematurely induced ovulation within 6.5 to 9.5 h of an intravenous injection of a luteinizing hormone preparation from horse pituitaries (Fraps, Riley & Olsen, 1942). Other gonadotrophins such as follicle-stimulating hormone (FSH) preparations and pregnant mare's serum (PMS) would also induce ovulation, though with less effect (Fraps, Olsen & Neher, 1942). The follicle destined to become the first egg in a sequence was comparatively more sensitive to ovulation-inducing gonadotrophins than were other follicles injected an equal time before a normally expected ovulation. The first ovulation of a sequence could be experimentally induced by as much as 20 to 24 h before the expected time and the responsiveness of all follicles was found to increase greatly as the interval between the previous ovulation and injection increased from 7 to 19 h (Fraps, 1967). The fact that the follicle destined to ovulate as the first of a sequence could be prematurely ovulated by 10 to 24 h suggests that the delay in ovulation which marks the end of a sequence cannot be attributed to the inability of this follicle to respond to LH. Neher & Fraps (1950) provided evidence to support this conclusion by injecting pituitary extract in order to induce ovulations onto the end of a normal sequence.

2. The ovulation-inducing hormone

By performing hypophysectomies at various intervals between

2 and 10 h prior to the expected time of ovulation, Rothchild & Fraps (1949a) demonstrated that a release of an ovulation-inducing hormone must occur 4 to 6 h before a normal ovulation for all members of a sequence. Supporting evidence was provided by Olsen & Fraps (1950), who found that changes began to occur in the nuclear membrane of the ovum 4.5 h before natural ovulation and ovulation prematurely induced by intravenous gonadotrophin injection.

Fraps, Fevold & Neher (1947) considered that the hormone initiating a normal ovulation is mainly or entirely LH, as mammalian LH induced ovulation in the adult hen. It was found that a fowl pituitary LH fraction was 500 times more potent as an ovulation-inducer than a fowl FSH fraction (Fraps, Fevold & Neher, 1947). Stockell-Hartree & Cunningham (1969) noted the similarity between mammalian and fowl LH in certain of their properties such as similarities in their ovarian ascorbic acid depleting activity and chromatographic behaviour, although Bullock, Mittal & Nalbandov (1967) have shown cross-reactions between fowl LH and mammalian LH antisera to be weak or absent. As chicken anterior pituitary powder was more effective than mammalian gonadotrophin in maintaining comb growth in hypophysectomized cockerels, Nalbandov, Meyer & McShan (1951) proposed that a third gonadotrophic hormone is present in the fowl pituitary. It has also been postulated that LH is released as a complex with FSH (Nalbandov, 1959; van Tienhoven, 1959).

Kao & Nalbandov (1972) proposed that LH may have to be altered in the bloodstream before it can act on the ovary, since they were able to induce ovulation by systemically injecting mammalian LH, but could not induce ovulation by injecting LH directly into the wall of

the follicle. However, a mixture of FSH and LH caused ovulation by this latter method. Clearly, this observation requires further investigation.

3. Involvement of gonadal steroids- a role for progesterone

The first evidence for an involvement of gonadal steroids in ovulation was given by Fraps & Dury (1943) who reported that doses of between 0.2 and 10.0 mg progesterone/hen subcutaneously administered 14 h before an expected ovulation would prematurely ovulate any egg in a sequence by 6 h. Fraps considered that progesterone may be the hormone directly involved in inducing natural ovulations. Rothchild & Fraps (1949b) demonstrated that progesterone failed to induce ovulation in the hypophysectomized hen and showed that the pituitary must remain intact for 2 to 4 h following progesterone injection for the consequent ovulation to occur. Rothchild & Fraps (1949b) thought it improbable that the release of LH could only take place 4 h after the progesterone injection since they surmised that progesterone would disappear rapidly from the bloodstream. Instead it was suggested that the release of ovulation-inducing hormone took place immediately following injection of progesterone. Other studies by van Tienhoven (1954) estimated the minimal duration of hypophyseal stimulation following progesterone injection to be 1.5 h. The period of time from progesterone administration to ovulation was found to be 7 to 11 h (Fraps & Dury, 1943), and, as this interval is not much longer than that required for ovulation following LH treatment, it supports the evidence showing an immediate action of progesterone on LH release. Rothchild & Fraps (1949a) observed that as the 7.5 h interval between

progesterone injection and consequent premature ovulation is the same for all follicles of a sequence, then the successively later hours at which ovulations occur within a sequence are due to later releases of the ovulating hormone.

In view of the restriction of ovulation to a limited period of the day, these authors suggested that LH release probably only occurs during the hours of darkness.

4. Neural involvement

Rothchild (1949) found that progesterone was no more effective in inducing ovulation when placed into the anterior pituitary than when applied at other sites including the thigh muscle and suggested that rather than acting directly on the pituitary, progesterone could stimulate LH release indirectly via some central nervous mechanism. This was confirmed in experiments where injections of 5 to 10 µg of progesterone into the pre-optic area of the hypothalamus caused premature ovulation; in contrast, similar injections into the anterior pituitary had no effect (Ralph & Fraps, 1960).

Other experiments which suggest a neural involvement in the control of ovulation are those showing the prevention of ovulation by injecting anti-adrenergic and anti-cholinergic drugs (Zarrow & Bastian, 1953) and the results of lesioning the brain. With regard to the latter, ovulation was interrupted for long periods by lesions in the paraventricular nucleus (Ralph, 1959), the median eminence and basal hypothalamus (Egge & Chiasson, 1963). Also lesions in the pre-optic region prevented progesterone-induced ovulation (Ralph & Fraps, 1959).

A neurohumoral link between the hypothalamus and pituitary

became apparent after it was found that severing the blood vessels connecting the anterior lobe of the pituitary to the median eminence resulted in regression of the ovary (Shirley & Nalbandov, 1956). Evidence of a gonadotrophin releasing factor in the hypothalamus was provided by the observation that an injection of extracts of chicken hypothalamus into the anterior pituitary caused premature ovulation (Clark & Fraps, 1967; Opel & Lepore, 1972). Also, Jackson & Nalbandov (1969a) extracted a substance from the fowl hypothalamus which stimulated a release of LH from rat anterior pituitary in an in vitro incubation system, and synthetic porcine LH-releasing hormone (LH-RH) has been shown to induce premature ovulations when intravenously injected into the laying hen (van Tienhoven & Schally, 1972).

After it had been established that interactions between the gonads, hypothalamus and pituitary are involved in controlling ovulation, three main hypotheses were formulated in the 1950s in an attempt to explain the hen ovulatory cycle.

Despite the findings of Fraps and co-workers that progesterone could induce premature ovulation, both Nalbandov (1959) and Bastian & Zarrow (1955) formulated hypotheses which disregarded any role for gonadal steroids in the control of LH release and ovulation.

5. Nalbandov's hypothesis

On the basis of the findings of Warren & Scott (1935) that ovulation does not generally occur while there is an egg in the oviduct, Nalbandov (1959) considered the possibility of an egg in the oviduct inhibiting a further ovulation by way of a neural link between the oviduct and the pituitary. This was supported by the observation that

a loop of thread secured in the magnum of the oviduct suppressed ovulation for up to 25 days without reducing the size of the ovary, comb and oviduct (Huston & Nalbandov, 1953). Nalbandov suggested that the passage of an egg through the magnum and isthmus neurogenically suppressed the secretion of the ovulation-inducing hormone. On clearing this part of the oviduct, levels of gonadotrophin in the circulation slowly recovered to pre-inhibition levels and, on reaching a critical level, ovulation was induced. He suggested that this period of recovery from neural inhibition would determine length of sequence; rapid recovery would result in shorter lag and therefore longer egg sequences, and a long period of recovery would result in shorter sequences. He accounted for the day of missed ovulation by suggesting that with each succeeding egg in a sequence the recovery rate would be slower, until at the end of a sequence the pituitary gland would not recover in time to cause ovulation of the next egg. However, there is experimental evidence to dispute this hypothesis. Opel (1965) reported that ovulation continues normally after transection of the oviduct, and it has been shown that when the egg in the uterus is prematurely expelled through manual crushing, the time of the next ovulation is not changed (Warren & Scott, 1935). Bullock & Nalbandov (1967) withdrew this hypothesis since they and others (Sykes, 1962; Lake & Gilbert, 1964) were unable to repeat the original findings of Huston & Nalbandov (1953).

6. Bastian and Zarrow's hypothesis

In view of the suggestion by Rothchild & Fraps (1949a) that the ovulatory release of LH could probably only occur during the hours

of darkness, Bastian & Zarrow (1955) proposed that increased activity associated with the hours of light prevents the release of LH, thereby confining it to a limited period of the 24 h day. They formulated a hypothesis to explain the later daily ovulations and the delay in ovulation which terminates a sequence. They suggested that there is a 24 h day-night rhythm of LH secretion interacting with an independent rhythmic maturation of ovarian follicles, and proposed that for about 8 h each night, even the night before a missed ovulation, an ovulatory stimulus (LH) is present. They proposed that follicles in a sequence mature at regular intervals of greater than 24 h and that if a follicle matured within the period when LH levels are elevated, it would ovulate. A follicle would mature at a later time each day until a stage would be reached when maturation occurred outside the period of high LH levels, and ovulation would be delayed until the next day. However, as Bastian & Zarrow pointed out, if follicles mature at a constant rate, the delayed follicle would ovulate at the beginning of an ovulatory period, although the succeeding follicle would be mature and be expected to ovulate at the same time the following day. This, therefore would not explain lag; so they suggested that follicular maturity is graded, and that a follicle of slightly less than full maturity could be ovulated provided that the ovulatory stimulus was present for a sufficient length of time. One of the implications of this hypothesis was that each follicle in a sequence should be ovulated at an earlier stage of development than its predecessor. This was supported by reports that the second and third eggs of short sequences were smaller than the first (Warren & Conrad, 1939; Bastian & Zarrow, 1955). Bastian & Zarrow also suggested that succeeding follicles in a sequence, being

progressively less mature, would therefore require a higher level of LH to ovulate them.

7. Fraps' hypothesis

The hypothesis of Fraps (1954) included a role for gonadal steroids in controlling ovulation. He suggested that there was a diurnal variation in the threshold of response of a neural component of the LH release mechanism to an "excitation hormone" which he suggested might be an ovarian progestogen. During the 8 to 9 h of the "open period" in which natural pre-ovulatory LH surges were assumed to be normally initiated, the neural threshold of sensitivity would be low. During the remaining 15 to 16 h of the ovulatory cycle, which Fraps referred to as the "closed period" when it was predicted that LH surges would not normally occur, the neural threshold of sensitivity would be high. As a follicle matured, the secretion of the "excitation hormone" from that follicle would increase until there was a sufficient concentration of the steroid in the blood to surpass the neural threshold of sensitivity, whereupon LH would be released and ovulation would occur. Since follicles mature at intervals of approximately 26 h, the formation of a mature follicle and hence production and/or release of sufficient "excitation hormone" to cause release of LH would occur later each day by the period of lag, so that eventually a stage would be reached when the follicle matured and produced a high concentration of "excitation hormone" at a time when the neural threshold was too high for LH release to occur, i.e. during the "closed period" of the ovulatory cycle. Only when the threshold was again lowered sufficiently would the "excitation hormone" produced by the mature

follicle stimulate a pre-ovulatory surge of LH.

Although Nalbandov's hypothesis was not supported by some existing experimental evidence, an adequate test of the hypothesis of Bastian & Zarrow or that of Fraps required the direct measurement of concentrations of LH and gonadal steroids in the blood. Parlow (1961) developed the ovarian ascorbic acid depletion (OAAD) bioassay for measuring LH in mammals, and this was used in several studies in which the secretion of LH was measured during the ovulatory cycle of the hen.

8. Measurement of LH using the OAAD bioassay

Direct measurement of LH during the ovulatory cycle of the hen using Parlow's (1961) OAAD bioassay suggested that there was more than one peak of activity. Measuring pituitary LH concentrations, Heald, Furnival & Rookledge (1967) and Tanaka & Yoshioka (1967) found two decreases during the ovulatory cycle, one soon after ovulation and the second 4 to 8 h before ovulation. The second decrease was postulated to reflect the pre-ovulatory surge of LH. Measuring plasma LH, Nelson, Norton & Nalbandov (1965) and Bullock & Nalbandov (1966, 1967) found three peaks of LH activity during the ovulatory cycle at 21, 13 to 14 and 8 h prior to ovulation. Bullock & Nalbandov were able to block all the peaks by administering dibenzyline, and suggested that a failure of LH release on the day of missed ovulation was due to a breakdown in the neural mechanism controlling the peaks of LH secretion. They suggested that the peaks at 20 and 13 or 14 h increased the sensitivity of the follicle to be ovulated, and that one of the peaks could be involved in the maturation of the succeeding follicle. Fraps (1967) suggested that the peak at 21 h before ovulation could be a maturational release of LH.

He observed that this peak preceded by about 4 h the time when the largest ovarian follicle could be first ovulated by an intravenous injection of chicken anterior pituitary powder, and may therefore stimulate the maturation of the follicle next due to ovulate. However, only one major peak of plasma LH, 5 to 7 h before ovulation, was found by Constantin (1969) using the same OAAD bioassay and in contrast to the findings of Heald, measured a small decrease in plasma LH just after ovulation.

Using the HCG-augmentation method of Steelman & Pohley (1953) a depletion in pituitary FSH was found to occur at about 11 h before ovulation (Kamiyoshi & Tanaka, 1969). Although FSH augments the ovulation-inducing actions of LH in the hen (Kamiyoshi & Tanaka, 1972) most evidence suggests that LH is the ovulation-inducing hormone (Fraps, Fevold & Neher, 1947).

9. Involvement of gonadal steroids other than progesterone in the control of ovulation

Before the development of radioimmunoassay, oestrone, oestradiol and oestriol had been identified in the ovary (Layne, Common, Maw & Fraps, 1958) and oestrone and oestradiol in the plasma of the laying hen (O'Grady & Heald, 1965).

A role for oestrogen in the control of ovulation was first suggested by Fraps (1954). Although intramuscular injections of oestradiol benzoate at dose levels of 0.2 to 0.5 mg/hen did not affect the timing of ovulation, doses of 1.0 to 2.0 mg/hen injected between 41 and 14 h before the first ovulation and between 28 and 11 h before the second ovulation delayed 14 % of the first and 55 % of second

expected ovulations in a sequence. Fraps suggested that the oestrogen injection delayed ovulation by decreasing the sensitivity or raising the threshold of the postulated neural component of the LH release mechanism to progesterone (see page 18). In the majority of these cases ovulation was delayed by 1 day until the hours during which ovulation would again normally occur. Fraps accounted for the greater incidence of delayed ovulation of the first egg in a sequence than of the second by suggesting that the neural threshold for LH release would be higher at the time of the first expected ovulation of a sequence than at the time of the second ovulation. He suggested that a small increase in the neural threshold of response caused by the oestradiol benzoate injection would result in failure of the second ovulation, while a much greater increase in the threshold would be required for the first ovulation in a sequence to be prevented. He suggested that in the normal situation, progesterone secreted by the mature follicle stimulates an increased release of LH which in turn increases the level of circulating oestrogen. This then raises the threshold of sensitivity of the neural component of the LH release mechanism. When the oestrogen levels falls, the threshold of the neural component falls and ovulation becomes possible.

Heald and co-workers also postulated a negative feedback role for oestrogen in the ovulatory cycle on finding that oestradiol benzoate injected at doses of 1.0 to 3.0 mg/kg increased the level of pituitary LH as measured by the OAAD bioassay (Heald, Rookledge, Furnival & Watts, 1968). They thought that an increase in pituitary LH levels measured between 8 and 12 h before a natural ovulation was

due to inhibition of LH release by increasing plasma levels of oestrogen, presumed to be secreted by the mature follicle. They suggested that LH release occurs when oestrogen levels in the blood fall. This view was supported by the observation that after an injection of [^{3}H]oestradiol, radioactivity accumulated in the pituitary and the amount taken up was greatest at 8 to 16 h before the predicted time of ovulation, although no accumulation of the steroid occurred in the median eminence and the hypothalamus (Hawkins, Heald & Taylor, 1969).

Growth and reddening of the comb in pullets has for a long time been attributed to androgen secretion (Ludwig & Boas, 1950; Arrington, Fox & Bern, 1956). When 1 mg testosterone/hen was injected 14 h before the predicted time of the first ovulation of a sequence, premature ovulations were observed in 13 of 32 (41 %) hens, compared with 18 of 19 (95 %) hens injected with progesterone at a similar time (Fraps, 1955b). However, Heald et al. (1968), using the OAAD bioassay, observed no change in the concentration of LH in the pituitary after injecting testosterone propionate or acetate at doses of between 0.1 and 1.0 mg/kg.

It is now apparent that observations on the secretion of LH using the OAAD bioassay must be viewed with caution, since this bioassay has been shown to be non-specific for chicken LH. OAAD activity was detected in the blood of adenohypophysectomized cockerels (Frankel, Gibson, Graber, Nelson, Reichert & Nalbandov, 1965) and Jackson & Nalbandov (1969b)detected OAAD activity in extracts of fowl pituitaries which had been boiled for 10 minutes, a treatment known

to destroy LH activity. Both studies suggested that arginine vasotocin was responsible for the OAAD activity.

In 1972, Follett, Scanes & Cunningham developed an homologous radioimmunoassay for avian LH which was sufficiently sensitive to measure LH in small samples (less than 100 µl) of plasma. Using this radioimmunoassay, Cunningham & Furr (1972) and Furr, Bonney, England & Cunningham (1973) subsequently reported only one peak of LH secretion during the ovulatory cycle between 4 and 7 h before ovulation. At about the same time sensitive radioimmunoassays and protein-binding assays were being developed to measure gonadal steroids in the circulation.

D. MEASUREMENT OF GONADAL STEROIDS DURING THE OVULATORY CYCLE

1. Progesterone

Using competitive protein-binding assays, Peterson & Common (1971) and Kappauf & van Tienhoven (1972) detected a maximal concentration of progesterone 2 to 6 h prior to the time of an expected ovulation while no rise was observed on the day of missed ovulation. This finding was confirmed by Haynes, Cooper & Kay (1973) using a double isotope derivative assay, and by Furr et al., (1973a) who, using a radioimmunoassay technique, found that progesterone levels in the blood rose shortly before or at the same time as LH. Furr et al., (1973a) found no evidence for a rise in progesterone levels starting after the beginning of the LH surge in samples taken at hourly intervals. Lague, van Tienhoven & Cunningham (1975) using 20 min sampling intervals observed that the increase in plasma LH

concentration may immediately precede that of progesterone, although the pre-ovulatory peaks of progesterone and LH secretion coincided. However, no definite relationship could be established. The absence of a progesterone peak on the day of missed ovulation (Furr et al., 1973a) is contrary to Fraps'hypothesis, as he suggested that a peak of progesterone secretion should occur on the day of missed ovulation since a mature follicle is present at that time (Fraps, 1954).

2. Oestrogen

A rise in plasma oestrogen levels has been detected by means of radioimmunoassay at 4 to 6 h prior to ovulation with a return to basal levels at or immediately following ovulation (Lague, 1972; Peterson & Common, 1972; Senior & Cunningham, 1974; Shodono, Nakamura, Tanabe & Wakabayashi, 1975). When Senior & Cunningham (1974) measured oestradiol and LH in the same plasma samples, it was found that oestradiol levels started to rise about 2 h before those of LH. In contrast, Lague et al., (1975) found that peaks of both LH and progesterone preceded peaks of oestrone and oestradiol.

3. Androgen

Both Peterson, Henneberry & Common (1973) and Etches (1974) measured androgen levels in the plasma throughout the ovulatory cycle and reported increases between 10 and 2 h before ovulation, with the peak of secretion occurring about 8 h before ovulation. Neither author observed a rise in androgen secretion on the day when no ovulation was expected. However, there appears to be a discrepancy concerning the timing of the testosterone peak in relation to that of LH. From the study of Etches (1974) it can be deduced that testosterone levels start

to increase about 2 h before those of LH. In contrast, Shahabi, Norton & Nalbandov (1975) reported a rise in the plasma level of testosterone at the same time before ovulation as a rise in progesterone, with both steroids reaching peak values at 4 to 5 h before ovulation. If, as Furr et al., (1973a), Lague et al., (1975) and Shodono et al., (1975) have found, progesterone rises at nearly the same time as LH, then it is possible that testosterone and LH are released almost concurrently.

When the concentrations of testosterone, oestrogen and progesterone were measured in the three largest ovarian follicles of a hierarchy, an increase in the follicular concentration of each steroid was found at about 19 h after the previous ovulation (Shahabi, Norton & Nalbandov, 1975). It is therefore apparent that synthesis of follicular steroids is concurrent with their pre-ovulatory release. However, no details in the study of Shahabi, Norton & Nalbandov (1975) were given for the synthesis of steroids on the day when an ovulation is delayed at the end of a sequence. While most of the progesterone is synthesized in the largest follicle, the increase in testosterone synthesis is inversely related to follicular size in the three most mature follicles in which the steroid levels were measured. Increased oestrogen synthesis was only observed in the smallest of the three follicles (Shahabi, Norton & Nalbandov, 1975). Senior & Furr (1975) found that the highest ovarian levels of oestradiol were in follicles of less than 5 mm diameter and in the ovarian stroma and that secretion of oestradiol ceased in maturing follicles between 50 and 20 h before ovulation.

It also appears that the release mechanism of these three steroids differs. Within 5 min of a single injection of 25 μg ovine LH, both follicular and plasma concentrations of testosterone and progesterone increased, while no change was observed in levels of oestrogen (Shahabi, Bahr & Nalbandov, 1975).

Although there is conflicting evidence as to the temporal relationship between the pre-ovulatory peaks of testosterone, oestradiol, progesterone and LH, it is apparent that rises in the blood levels of each of these hormones occur at approximately the same time and it seems important to re-investigate the feedback mechanisms by which gonadal steroids regulate the secretion of LH. This is the purpose of the present study.

MATERIALS AND METHODS

I. EXPERIMENTAL HENS AND THEIR MAINTENANCE

The experiments described in this study were performed on domestic fowl of either a strain derived from the Rhode Island Red (Thornber 909) or of a strain derived from the White Leghorn (Shaver). They were hatched at the Stock Farm, Roslin, and transferred as day-old chicks to the brooder at the Poultry Research Centre, Edinburgh. The temperature in the brooder room was regulated at 68 to 72° F and a lighting schedule of 14 h light : 10 h darkness (lights on 07:00 - 21:00 h) was used. Feed was commercial chick mash. At 8 weeks, the fowl were transferred to a windowless, air-conditioned battery house where a similar temperature and lighting schedule were maintained. The fowl occupied individual cages of dimensions [46 x 30 x 40] cm and were allowed free access to water and food (commercial Farm Flock layers pellets). Most cages were fitted with automatic ovipositing recording devices which recorded ovipositions to the nearest minute.

II. SURGICAL PROCEDURES

A. COMPLETE OVARIECTOMY

Ovariectomies were performed on immature birds of between 10 and 14 weeks of age, i.e. 4 to 8 weeks before the start of follicular growth.

Thirty minutes before the operation, each pullet was injected into the breast muscle with about 0.6 ml Rompun solution (2 %) per kg body weight (Bayer Agrochem. Ltd., Suffolk). This caused muscular

relaxation and sedation.

At the start of the operation, 0.2 to 0.3 ml of Veterinary Nembutal (Abbott Laboratories, Kent) was injected into the brachial vein and 2 to 3 min were allowed for the anaesthetic to take effect. If necessary, further intravenous injections of 0.05 ml each were administered until an appropriate plane of surgical anaesthesia was achieved. Thereafter, similar doses were given whenever there were signs of recovery from the anaesthetic.

The anaesthetized pullet was laid on its right side and the legs and left wing were secured to the edges of the operating table with surgical tape. Feathers were plucked from the area about the site of the proposed incision and the exposed skin was wiped with cotton wool soaked in 70 % alcohol. Using fine-pointed scissors, an incision of about 5 to 7 cm was made in the skin over the last two ribs. The exposed thigh muscles were tied back with Ethicon Mersilk suture in order to gain access to the last two ribs. Throughout the operation these thigh muscles were kept moist with 1 % saline. An incision was made through the intercostal muscles between the last two ribs and, taking care not to fracture either of the ribs, they were held apart by retractors. This exposed the body cavity and, by perforating the abdominal air sacs, the ovary could be seen ventral to the cephalic lobe of the kidney and overlying the posterior vena cava. In these pullets of 10 to 14 weeks of age, the ovary appeared as a flat mass of microscopic (< 1 mm diam) follicles and medullary tissue enclosed within the peritoneal membrane, and attached to the body wall by a short mesovarium. Using fine-pointed straight forceps, the peritoneal covering was removed and the perimeter of the ovary

eased away from the body wall. Care was taken to prevent rupture of the vena cava or left iliac vein and to avoid damaging the adrenal gland which was embedded in the underlying surface of the ovary. Small pieces of ovarian tissue were then removed with forceps until only the mesovarium remained. Using an electro-cautery unit, this was seared and the surface of the surrounding area was cauterized to destroy any remaining ovarian tissue. During this procedure, the body wall was kept moist by occasionally swabbing with cotton wool soaked in 1 % saline. In the event of rupturing a large blood vessel, bleeding was controlled by applying either Sterispon no. 4 (Allan & Hanburys Ltd.) or Thrombin powder (Parke-Davis).

Following removal of the left ovary, the ribs were sutured (Dexon absorbable sutures) and, after spraying Aureomycin powder, a strip of Sterispon was placed over the site of incision. The skin was then sutured and Aureomycin powder again sprayed over this area.

The pullet was then laid on its left side and the rudimentary (right) gonad located by the same means. This was barely visible as a pale film overlying the vena cava and situated next to the right adrenal gland. The right gonad was electro-cauterized and the same procedure followed as before. The operation of ovariectomy took between 1 and 1½ h and the pullets generally regained consciousness within 1 h of completion.

In completely ovariectomized pullets the comb remained undeveloped and pale and the new plumage resembled that of a cockerel. Frequently, however, the comb and wattles began to grow and redden several weeks after the operation. In these cases the pullets were again laparotomized and any gonadal growth, which was usually of the

rudimentary gonad, was removed. If at 30 weeks of age there was no sign of comb development and plasma LH levels were above 30 ng/ml, then a hen was considered fully ovariectomized and suitable for experimentation.

B. REMOVAL OF OVARIAN FOLLICLES

One experiment required the removal of all ovarian follicles of over 5 mm diameter from hens previously laying regular sequences of between 4 and 10 eggs. Access to the ovary was gained using the same surgical procedure as described for complete ovariectomy. Since the large yolky follicles are well-vascularized, it was necessary to clamp the follicular stalk with artery clamps before cutting away the ovarian follicle. The excised ovarian follicle was carefully eased out of the body cavity through the last two ribs which were held apart using retractors. The clamp was left in place for 2 or 3 minutes while other follicles were similarly removed. The removal of the follicles of less than 10 mm in diameter did not require the application of a clamp as bleeding was slight. After careful examination of the ovary to check that no follicles of over 5 mm in diameter remained, the ribs and skin were sutured separately.

Control hens were treated similarly, but the ovarian follicles were handled instead of being removed.

III. EXPERIMENTAL PROCEDURES

A. ADMINISTRATION OF GONADAL STEROIDS

All steroids were administered by injection into the breast muscle. They consisted of the following :- analar oestradiol-17β, oestradiol benzoate, oestrone, testosterone, androstenedione, progesterone and the adrenal steroid deoxycorticosterone. The solvent for the free steroids was either 1 : 1 propylene glycol and ethyl alcohol acidified with 1 drop of 0.1 M hydrochloric acid/4 ml solution, or arachis oil, and each steroid was injected according to body weight in a volume of 0.3 to 0.5 ml.

B. ADMINISTRATION OF ANTISERA TO GONADAL STEROIDS

The antisera to oestradiol-17β, progesterone and testosterone were all kindly donated by Dr R.J. Scaramuzzi of the M.R.C. Unit of Reproductive Biology. The antisera had been raised in sheep and each had been shown to specifically bind the appropriate tritiated antigen (R.J. Scaramuzzi, personal communication). The undiluted antisera were injected into the brachial veins of regularly laying hens at a dose of 1.25 ml/kg body weight.

C. ADMINISTRATION OF LH-RELEASING HORMONE

The LH-releasing hormone was gifted by Mr J.M.J. Best of Hoechst Pharmaceuticals (Veterinary Division) and the same batch of synthetic LH-RH was used in all experiments. It was dissolved in 1 % saline and stored frozen at a concentration of 40 μg/ml until required for use when it was further diluted with 1 % saline. The LH-RH was

injected into the brachial vein and used at a concentration of 10 μg/kg for intact hens and 1 μg/kg for ovariectomized hens.

D. COLLECTION OF BLOOD SAMPLES

Blood samples were collected from the brachial veins of unanaesthetized fowl using either a cannulation technique or venepuncture. When samples were collected during the night, the hens remained in darkness except when a sample was actually being taken. For this, the hens were carried from the cages 20 yards to a table at the end of the room where a lamp with a 21 watt bulb, covered in green cellophane, provided just sufficient light to enable blood samples to be taken.

1. Cannulation

After testing cannulae of varying diameters and flexibility, and several types of needles, suitable equipment was considered to be 40 cm lengths of single lumen polyethylene catheters (I.D., 0.45 mm; O.D., 0.75 mm; Dural Plastics & Engineering Pty Ltd, New South Wales) and 1½ in, 18-G Thinwall Needles (Abbott Laboratories, Kent).

Before use, the interior of each cannula and needle was rinsed with heparin (Pularin Heparin injection, B.P. 5000 U/ml) diluted to 50 U/ml with 1 % saline. The experimental hen was restrained by tying both legs together and, with the animal laid on its left side, the right wing was held open and the needle inserted into the brachial vein at the elbow joint of that wing. One end of the cannula was then passed through the needle into the vein and towards the heart until the tip was estimated to reach the anterior vena cava. The needle was then withdrawn from the vein and from the cannula. A luer-fitting hub

which had been cut from a Nylon Intravenous Cannula Set (Portex Ltd., Kent) was joined to the free end of the cannula by a short piece of plastic tubing, and a heparinized disposable polypropylene syringe was connected to it. A blood sample was immediately withdrawn to check that the blood could flow easily. If necessary, the cannula was adjusted slightly until functioning correctly. Using a separate syringe, after each withdrawal of a blood sample between 0.1 and 0.2 ml of diluted heparin was injected through the cannula to prevent clotting. Care was taken to prevent the injection of air bubbles with the heparin. The syringe and luer-fitting cannula hub were then removed and the open tip of the tubing was heat-sealed using a soldering iron. To secure the cannula, it was taped to the wing with 4 cm strips of elastoplast. When further blood samples were withdrawn, the first 0.2 ml was discarded to remove the heparin and a further 0.5 to 0.8 ml was then taken for assay.

Where the intervals between sampling were greater than 10 min, the red blood corpuscles were periodically re-injected through the cannula, having first been resuspended in an equal volume of 1 % saline and then warmed to body temperature. During experiments involving 5- and 10-min sampling, red blood corpuscles were not re-injected, as it was thought that this procedure might have a short-term effect on the release of LH.

Throughout the sampling period, the fowl were unrestrained and allowed free access to food and water.

This method of sampling enabled the withdrawal of many blood samples at frequent intervals.

2. Venepuncture

The method of sampling blood by separate punctures of the brachial vein was advantageous over the method of cannulation in two ways. First, it did not require the pre-experimental procedure of cannulation and, secondly, the time taken in withdrawing a sample was shorter. The main disadvantage of this method was the limited number of samples that could be obtained by separate punctures of the vein due to the development of haematomas. However, with care, 12 or more samples could be withdrawn at 15- or 30-min intervals, sufficient for the requirements of most of the experiments. To take a blood sample, the hen was restrained by tying both legs together; samples of between 0.75 and 1.0 ml were withdrawn from a wing vein into heparinized 2 ml syringes with 25-G hypodermic needles.

When samples were required at 2-min intervals for an experiment of short duration, e.g. 16 min, the hen was restrained by the legs and throughout the experiment the needle was left in the vein of the wing which was held open. As the sampling intervals were very frequent, blood was seldom found to clot inside the needle. This method was useful in that it incorporated a single puncture of the vein, thereby reducing the risk of haematomas. However, this method was only appropriate when using very short sampling intervals and, unlike the other methods of sampling, necessitated the use of one other person apart from the experimenter in order to continually restrain the hen in such a position that the needle did not become displaced.

Whichever method of blood sampling was used, all samples were collected into heparinized 2 or $2\frac{1}{2}$ ml disposable syringes and

immediately transferred to 60 x 10 mm polystyrene tubes, each containing one drop of heparin (50 U/ml). The blood was then centrifuged at 1800 g for 4 min and the plasma was separated and stored at -20° C in stoppered 44 x 9 mm polystyrene tubes until required for assay.

IV. MEASUREMENT OF PLASMA LH CONCENTRATION USING RADIOIMMUNOASSAY

The concentration of luteinizing hormone in the plasma was estimated using a post-precipitation double-antibody radioimmunoassay procedure developed and described for avian LH by Follett, Scanes & Cunningham (1972).

A. DILUENT FOR STANDARDS AND PLASMA SAMPLES

The diluent consisted of the following reagents:-
0.05 M-sodium phosphate buffer, 0.15 M-sodium chloride, 0.1 % sodium azide, 0.01 M-ethylenediaminetetra-acetic acid and horse serum no. 5.

The 0.05 M-sodium phosphate buffer was made up of 250 ml 0.5 M-disodium hydrogen phosphate anhydrous (70.98 g/l) with 0.5 M-sodium dihydrogen phosphate dihydrate (78.0 g/l) added until the pH reading was 7.5. About 35 ml of sodium dihydrogen phosphate was required.

To 160 ml of the 0.5 M-sodium hydrogen phosphate buffer was added 800 ml distilled water, 17.5 g of sodium chloride, 5.84 g of ethylenediaminetetra-acetic acid, 2.00 g of sodium azide and 40 ml of horse serum no. 5 (Wellcome Reagents). The diluent was stirred until all reagents had dissolved and was then made up to 1800 ml with

distilled water. The pH, which was then about 6.0, was adjusted to 7.0, using approximately 48 ml of N sodium hydroxide, and distilled water was added to make the diluent volume up to 2000 ml.

B. THE PURIFIED LH

The purified LH was the fraction AE1 donated by Dr. B.K. Follett and Dr C.G. Scanes of the university College of North Wales, Bangor. The LH had been extracted from the pituitaries of broilers and laying hens, using the method of Stockell-Hartree & Cunningham (1969), and then purified to minimize TSH activity (Scanes & Follett, 1972).

C. IODINATION OF AVIAN LH

The purified LH was labelled with $Na^{125}I$ obtained from the Radiochemical Centre, Amersham, according to the method of Greenwood, Hunter & Glover (1963).

D. ANTISERA

The antisera used in these studies were raised in rabbits and were either CM2 15/8, donated by Dr C.G. Scanes and Dr P.J. Sharp, or IRC2 (terminal bleed) donated by Dr F.J. Cunningham (University of Reading). The IRC2 was the more sensitive of the two antisera but the limited quantity available restricted its use to only a few of the experiments described in this study. The antisera were used at initial dilutions of 1 : 20,000 (CM2 15/8) and 1 : 2,500 (IRC2/T). These concentrations had been estimated to bind 30 - 50 % of ^{125}I-labelled LH in the absence of unlabelled LH.

E. STANDARDS

The standard AE1 was used at concentrations estimated to compete equally with the labelled LH for binding sites on the antiserum. Hence the standard was used at a different range of concentrations for incubating with each antiserum.

1. Incubation with anti-IRC2

The purified chicken LH was stored at -20° C in a concentration of 1250 ng/ml. When required, 100 µl was diluted to 7000 µl to give a concentration of 17.85 ng/ml (i.e. 3.57 ng/tube of 200 µl). Eight standards were obtained by serial $\frac{3}{4}$ dilutions in volumes each of 200 µl.

2. Incubation with anti-CM2 15/8

The LH was frozen at 210 ng/ml and when required was diluted to 7.00 ng/ml (i.e. 1.40 ng/tube of 200 µl) and $\frac{3}{5}$ serial dilutions were carried out to provide 8 standards of 200 µl each.

F. RADIOIMMUNOASSAY METHOD

The assay consisted of :-

i) Three "total count" tubes containing 8000 counts of ^{125}I-labelled LH only. These provided an estimate of the unbound labelled LH.

ii) Three "blanks" containing all reagents except antisera and unlabelled LH. This indicated the amount of background binding of labelled LH.

iii) Six "zero standard" tubes containing all reagents except unlabelled LH. The counts in these tubes represented 100 % binding of the antiserum to unlabelled LH.

iv) Twenty-four tubes containing the 8 standard known concentrations

of purified unlabelled LH (in triplicate) and all other reagents. The counts from these tubes expressed the binding of labelled LH to the antisera in the presence of specified concentrations of unlabelled LH. They were expressed as a percentage of the "zero standard" count (after subtracting the background count) and were used to calculate the calibration curve.

v) Nine tubes containing 3 dilutions (in triplicate) of the control plasma sample required for estimating inter-assay variation.

vi) A variable number of tubes containing test samples at 1 dilution in triplicate, or at 3 dilutions in duplicate.

Unless otherwise stated, samples were assayed at 1 dilution in triplicate. The concentrations of LH in samples taken from individual hens in a single experiment were all measured in the same assay.

Test samples were diluted and dispensed at the required concentration using either a Micro-medic Automatic Pipette (Model 25002, Chem Lab Instruments Ltd.) or a Compu-pet 100 (William R. Warner & Co.Ltd.). The following dilutions were used :-

For incubating with anti-IRC2 :-

Not injected with steroids	Intact female	- 30 to 60 µl in 200 µl
	Intact male	- 10 to 15 µl in 200 µl
	gonadectomized male or female	- 2 µl in 200 µl

For incubating with anti-CM2 15/8 :-

Not injected with steroids	Intact female	- 50 to 100 µl in 200 µl
	Intact male	- not applicable
	Gonadectomized male or female	- 5 to 10 µl in 200 µl

These dilutions were estimated to generally bind in the region of maximum precision on the calibration curve, i.e. the region of 50 % binding.

On the day of assay, plasma samples were diluted to the required concentration in 200 µl of diluent and dispensed into 50 x 6 mm disposable polystyrene tubes. 200 µl of diluent were dispensed into the 3 "blanks" and the 6 "zero standard" tubes. 50 µl of the antiserum which had been diluted to the required concentration were added, using a $2\frac{1}{2}$ ml Hamilton Repeating Dispenser, to all tubes except the "blanks" and "total count" tubes. The contents of the tubes were mixed using a rotamixer and then incubated for 24 h at 4° C.

On the second day, 50 µl of ^{125}I-labelled LH was added to all assay tubes and to 3 "total count" tubes, at a concentration calculated to provide 8000 counts per tube. This volume was added with a separate Hamilton Dispenser used only for this step in the assay procedure. The contents of the tubes were mixed and then incubated for 24 h at 4° C.

On the third day, 50 µl of normal rabbit serum (1 : 200) (Wellcome Reagents) were added to each tube, except the "total count" tubes, followed by 50 µl of precipitating antiserum (Donkey-Rabbit Precipitating Serum 1 : 20, Wellcome Reagents). The contents of each tube were again mixed and then incubated for a further 24 h at 4° C.

On the fourth day, the tubes were centrifuged for 40 min at 2500 rev/min and 4° C and the supernatants were discarded, leaving the precipitated antigen-antibody complex in the bottom of each tube to be counted for 100 s on a Wallac 80000 Gamma Sample Counter (L.K.B. Instruments Ltd., Surrey).

G. TREATMENT OF RADIOIMMUNOASSAY DATA

The bound : free ratios in each assay could be calculated by comparing the counts in the 3 "blank" tubes (containing labelled LH and no antibody) with those in the 6 "zero standard" tubes (containing labelled LH and antibody).

The amount of labelled LH bound to the antibody in the absence of unlabelled LH was considered to represent 100 % binding and all results were expressed as a percentage of this figure. The amount of ^{125}I-labelled LH bound to the anti-LH was reduced as the amount of unlabelled LH was increased in the form of a sigmoid curve. The sensitivity of the assay was estimated by the quantity of unlabelled LH (AE1) required to reduce the binding to 50 %; it varied with the antisera and iodinated LH preparation.

For most of the experiments described in this study, potency estimates, together with their 95 % confidence limits were calculated using a computer programme developed by Rodbard and Lewald (1970) and modified as a Radioimmunoassay Data Processing Programme Package by Mr W.R. Carr, Mr D. Maxwell and Mr D.T. Wilson of the A.R.C. Animal Breeding Research Organization, Edinburgh. Potency estimates for other experiments were obtained by manual calculation though, in these cases, the 95 % confidence limits for each estimation could not be calculated.

The computer programme was designed for use with a double antibody radioimmunoassay which linearizes the standard curve using logit-log transformation. The programme calculates a calibration curve using logit-log transformation and iterative least squares regression analysis. By reference to the calibration curve, the programme provides the mean estimates and 95 % confidence limits for "unknown" and "control" samples based on the pooled residual variance of the standard curve and the sample. Where replicates are of more than one concentration, the calculation of a regression line enables the sample to be tested for linearity and parallelism with the calibration curve. It also compares selected parameters of the current assay with previous assays and examines between-assay variance. An estimate is provided of the minimum detectable dose; this is a measure of the sensitivity of the assay and in the programme is defined as "the smallest dose for which the response is significantly different from the zero dose value".

The flexibility of the system allows for an undefined number of replicates within the "total", "blank", and "zero standard" and combinations of up to 9 replicates at each of 9 concentration levels for each "unknown" sample.

H. PRECISION AND SENSITIVITY OF THE RADIOIMMUNOASSAY

Two-hundred and seven radioimmunoassays were required to complete this study; in 140 assays, the computer programme was used to calculate LH values.

Seven different pools of plasma were used as controls. Mean LH values for 4 pools of plasma from 15 to 24-week-old pullets were

4.78 ± 0.47 (S.D.) ng/ml (n = 14) (range 4.28 to 5.96 ng/ml), 4.69 ± 0.87 ng/ml (n = 31) (range 3.05 to 5.84 ng/ml), 3.48 ± 0.46 ng/ml (n = 16) (range 2.68 to 4.03 ng/ml) and 3.48 ± 0.66 ng/ml (n = 28) (range 2.77 to 4.95 ng/ml). Mean LH values in 2 pools of plasma from ovariectomized hens were 44.84 ± 3.42 (S.D.) ng/ml (n = 13) (range 40.0 to 51.4 ng/ml) and 37.0 ± 5.29 ng/ml (n = 21) (range 30.1 to 47.5 ng/ml) and the mean LH value for a pool of cockerel plasma was 11.1 ± 1.19 (S.D.) ng/ml (n = 17) (range 10.3 to 14.8 ng/ml).

The minimum detectable dose of LH was calculated by computer programme for 54 assays using CM2 15/8 antiserum and ranged from 0.013 to 0.475 ng/ml (mean, 0.12 ± 0.10 (S.D.) ng/ml).

Statistical calculations

Unless otherwise stated, Student's t-test was used for the statistical calculations.

RESULTS

I. PATTERNS OF LH SECRETION IN THE DOMESTIC FOWL

Before examining the effects of gonadal steroids on LH secretion, it was considered appropriate to determine the normal patterns of LH secretion in fowl and to what extent individual plasma LH levels are representative of mean circulating concentrations.

A preliminary study involving hourly blood sampling from the wing veins of three Shaver cockerels by means of venepuncture, showed that levels of plasma LH fluctuated during the day by as much as 38 to 52 % from individual mean levels of 7.7, 8.2 and 11.5 ng/ml.

Recent studies in several mammalian species have shown that over short periods, similar wide fluctuations in plasma LH levels within individuals are the result of the hormone being released into the circulation in rapid secretory episodes (see page 133).

The possibility that LH is secreted episodically in the domestic fowl was therefore investigated by following more closely the changes occurring in the level of circulating LH within intact cockerels, intact hens and gonadectomized fowl. Serial blood samples were taken at intervals of between 10 and 30 min through cannulae as described on page 32. In order to determine if inserting the cannula into the wing vein affected LH secretion, blood sampling was started in some birds immediately after cannulation, and in others after intervals of 4 to 18 h post-cannulation. Plasma LH levels were measured using the radioimmunoassay method described on page 37. Each sample was assayed in duplicate at 3 dilutions and each series of blood samples was assayed in one assay.

A. INTACT COCKERELS

When blood samples were taken from 11 cockerels (7 Thornbers and 4 Shavers) at 30 min intervals for a period of 12 h, two birds showed a rapid drop in plasma LH levels to a steady 2 to 3 ng/ml, whereas the LH levels in the other 9 birds, despite an immediate depression, showed fluctuations which took the form of episodic discharges. The LH patterns of 4 such birds are shown in Fig. 1. Each of these secretory episodes lasted 1.5 to 2 h and was characterized by a rapid rise and slower fall in plasma LH levels. 95 % confidence limits around each plasma LH value distinguished secretory episodes from some smaller fluctuations that could have been due to intra-assay error.

A more detailed examination of secretory episodes in 4 birds, using 10 min sampling for a period of 3 h showed that in 3 of the birds a steep 100 to 200 % increase in plasma LH levels took place over 10 to 15 min followed by a more gradual decline forming an exponential curve (Fig. 2a, b, c). One bird (Fig. 2d) failed to show an episodic discharge of LH during the sampling period and it was noticeable that this had the lowest LH level of the 4 birds.

Several days after the completion of these experiments, 3 blood samples were taken at 3 to 4 h intervals by venepuncture from all the intact cockerels. The mean plasma LH values obtained for each of the cockerels by averaging the 3 estimations ranged from 7 to 16 ng/ml. Invariably when blood samples were taken at 30 or 10 min intervals, plasma LH levels either fell below this level during the initial sampling period (Fig. 1 and 2c, d) or were depressed at the start of

Figure 1

Variations in the concentration of LH in plasma samples taken from four intact cockerels at intervals of 30 min. Vertical lines represent 95 % confidence limits.

Figure 2

Variations in the concentration of LH in plasma samples taken from four intact cockerels at intervals of 10 min. Vertical lines represent 95 % confidence limits.

Fig.1

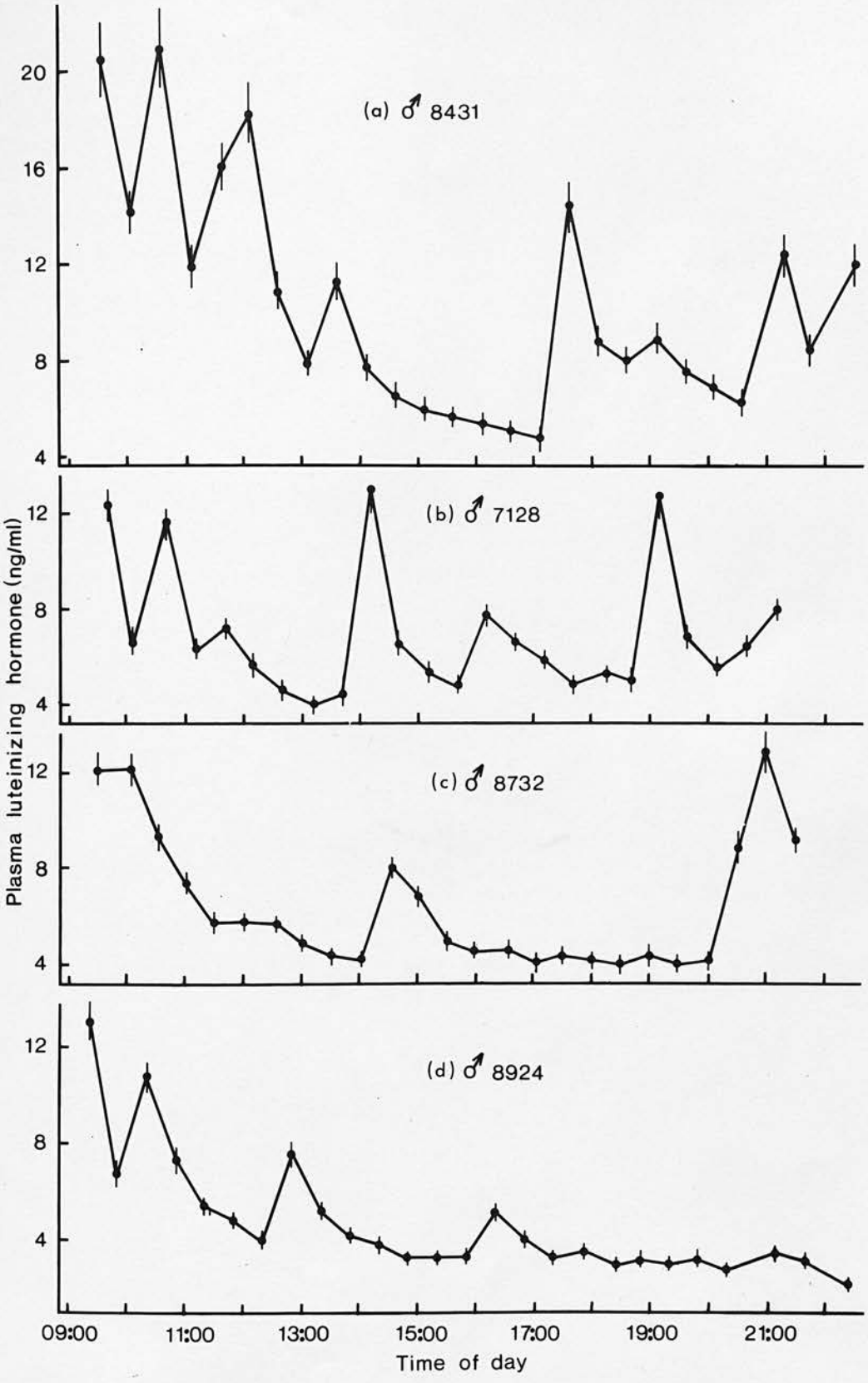
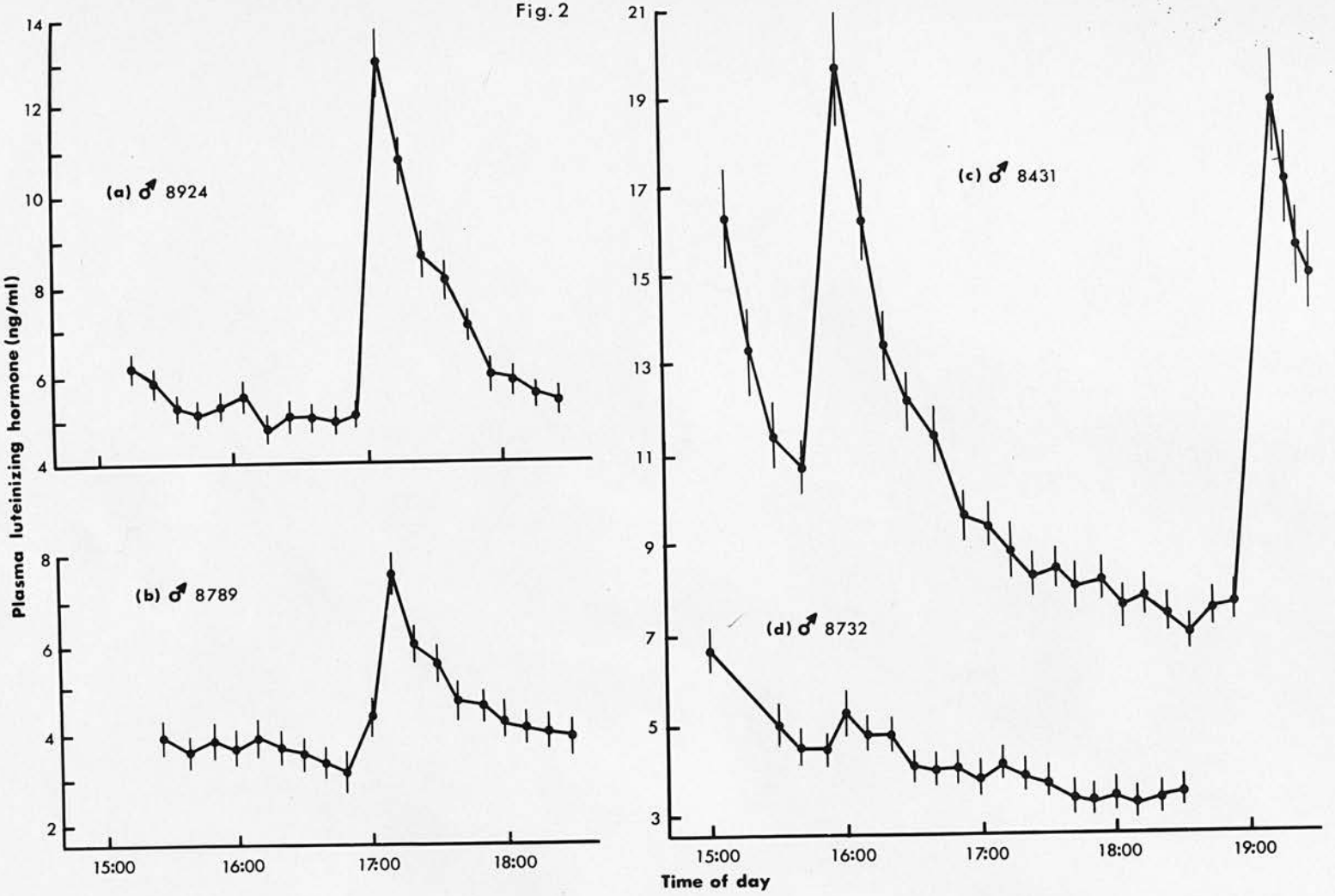

(a) ♂ 8431
(b) ♂ 7128
(c) ♂ 8732
(d) ♂ 8924
Plasma luteinizing hormone (ng/ml)
20
16
12
8
4
09:00
11:00
13:00
15:00
17:00
19:00
21:00
Time of day

Fig. 2


(a) ♂ 8924
(b) ♂ 8789
(c) ♂ 8431
(d) ♂ 8732
Plasma luteinizing hormone (ng/ml)
Time of day
15:00
16:00
17:00
18:00
19:00

sampling (Fig. 2a, b). This phenomenon was not entirely due to the process of cannulation, since it occurred in birds that had been cannulated 4 to 18 h before blood samples were taken (e.g. Fig. 2b, c, d). The extent to which plasma LH levels were depressed during frequent sampling varied from bird to bird. As plasma LH levels became more depressed, the episodic discharges of the hormone tended to become smaller (Figs. 1d and 2b) and in the birds with the most depressed LH values (2 to 3 ng/ml) they could not be detected.

No relationship could be established between the pattern of LH secretion and feeding, reinjection of red blood corpuscles or time of day.

B. GONADECTOMIZED FOWL

Blood samples were taken from gonadectomized birds (Thornbers) at either 20 to 30 min intervals for 12 h (5 females and 4 males), 10 to 15 min intervals for 3 to 6 h (2 females and 3 males) or 5 min intervals for 3 h (1 female and 1 male).

Mean levels of LH in the circulation of gonadectomized male and female fowl were of the same order of magnitude, ranging from 35 to 52 ng/ml (38.1 $\pm$ 2.5 (S.E.M.) ng/ml (n=8)) in males and from 30 to 77 ng/ml (43 $\pm$ 5.4 ng/ml (n=8)) in females. They were raised fourfold over the mean LH values in intact cockerels and 15 to 30-fold over the mean values in laying hens.

Concentrations of LH in plasma samples taken at 20 to 30 min intervals fluctuated in a random manner (Fig. 3) suggesting that any episodic release must have the periodicity of less than 30 min. This was borne out in experiments involving withdrawal of blood every 5 or

Figure 3

Variations in the concentration of LH in plasma samples taken from three ovariectomized hens and two castrated cockerels at intervals of 20 to 30 min. Vertical lines represent 95 % confidence limits.

Figure 4

Variations in the concentration of LH in plasma samples taken from three ovariectomized hens and four castrated cockerels at intervals of 5 min (A190, 8071), 10 min (9144, 7985, 8613, 8504) or 15 min (7871). Vertical lines represent 95% confidence limits.

Fig. 3

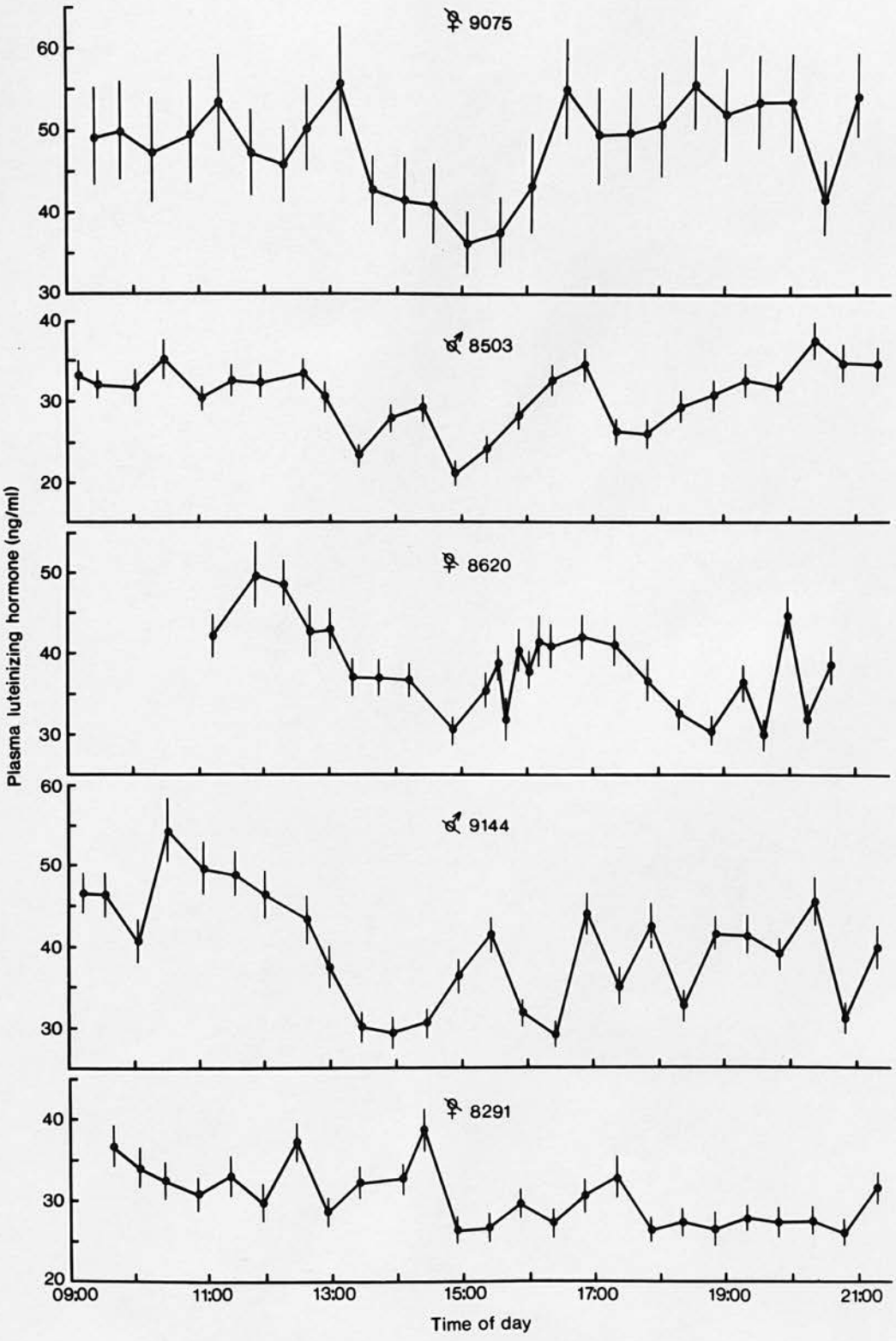
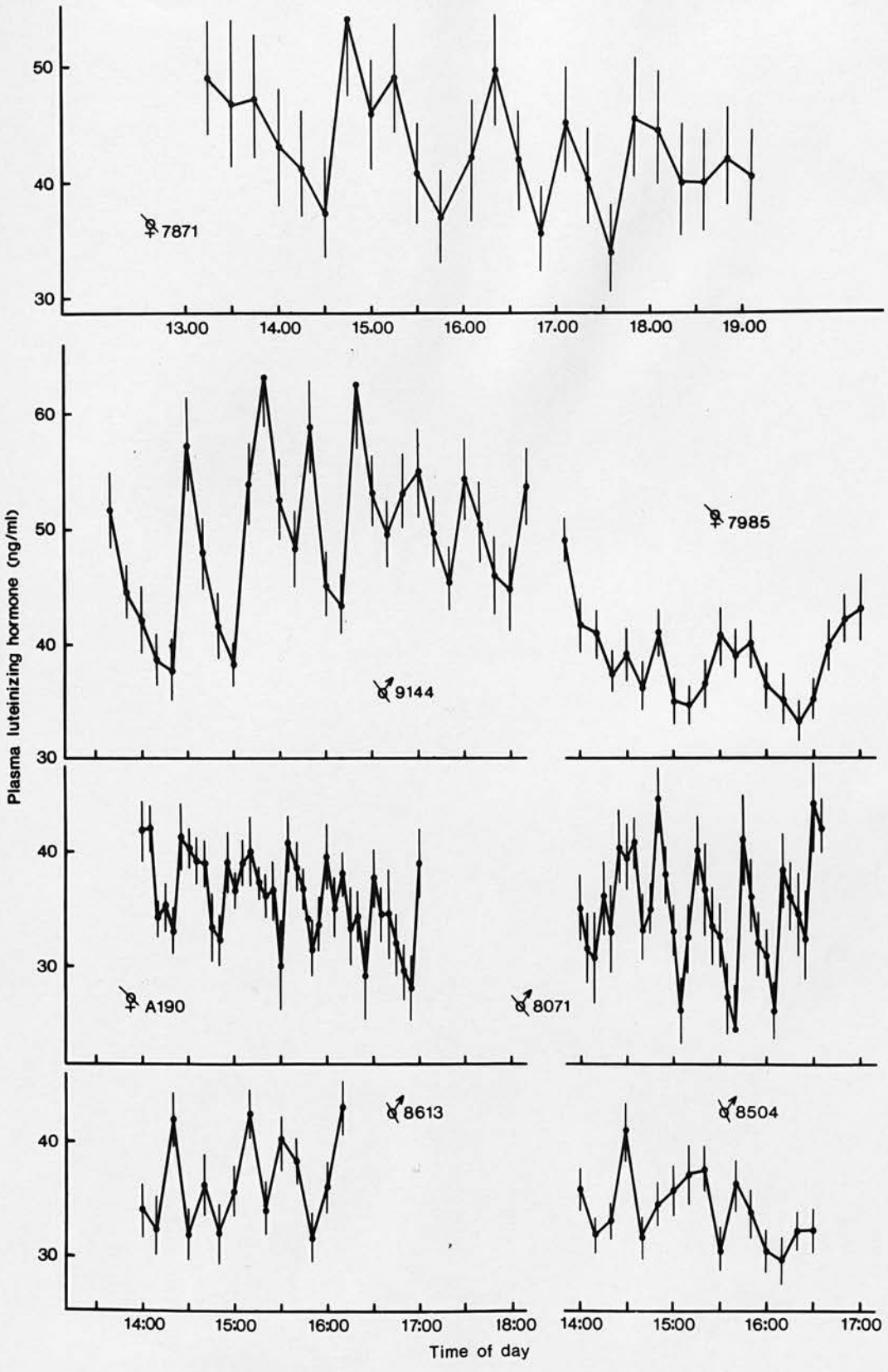

♀ 9075
♂ 8503
♀ 8620
♂ 9144
♀ 8291
Plasma luteinizing hormone (ng/ml)
09:00
11:00
13:00
15:00
17:00
19:00
21:00
Time of day

Fig. 4


♀7871
♀7985
♂9144
♀A190
♂8071
♂8613
♂8504
Plasma luteinizing hormone (ng/ml)
Time of day
13.00
14.00
15.00
16.00
17.00
18.00
19.00
14:00
15:00
16:00
17:00
18:00

10 min where fluctuations in plasma LH levels showed that the hormone was being released episodically at 20 to 45 min intervals (Fig. 4). This episodic release was three to four times more frequent than in intact cockerels (cf. Figs 4 and 1). As in cockerels, each secretory episode was initiated by a steep rise in LH levels over a 10 to 15 min period. This rise was in the order of 20 to 60 % which is considerably smaller than the 100 to 200 % rises encountered in the cockerels. The decay phase was shorter than in the cockerels, being interrupted by the next episodic discharge. In the case of one bird sampled at 10 min intervals (Hen 7985, Fig. 4) an episodic pattern of LH secretion was not clearly seen. This could have been due to stress since the LH levels were falling during the initial sampling period. Generally, however, there was little evidence that plasma LH levels were depressed by handling in gonadectomized fowl.

C. LAYING HENS

Blood samples were taken from one laying hen (Thornber) at 30 min intervals for 8 h and from five others at 10 min intervals for between 2 and 3.5 h; in one of these birds, the descending part of the pre-ovulatory LH peak was included in the sampling period.

The mean concentration of plasma LH in the six laying hens ranged from 1.5 to 2.4 ng/ml. When blood was sampled from one hen at 30 min intervals, small, though significant fluctuations were evident (Fig. 5b). However, at this frequency of sampling, no characteristic episodic pattern of secretion was discernible. Sampling from five hens, at 10 min intervals, likewise failed to demonstrate discrete secretory episodes, even when part of the descending slope of the pre-ovulatory

Figure 5

Variations in the concentration of LH in plasma samples taken (a) from a hen during the descending slope of a pre-ovulatory LH surge at intervals of 10 min and (b) from a hen at intervals of 30 min during a portion of the ovulatory cycle not including the pre-ovulatory LH surge. Vertical lines represent 95 % confidence limits.

Figure 6

Variations in the concentration of LH in plasma samples taken at intervals of 10 to 15 min from 15-week-old (□---□) and 20-week-old (●——●) immature hens. The cannulae were inserted at 16:00 h on Day 1 and serial sampling commenced at 10:00 h on Day 2.

Fig. 5

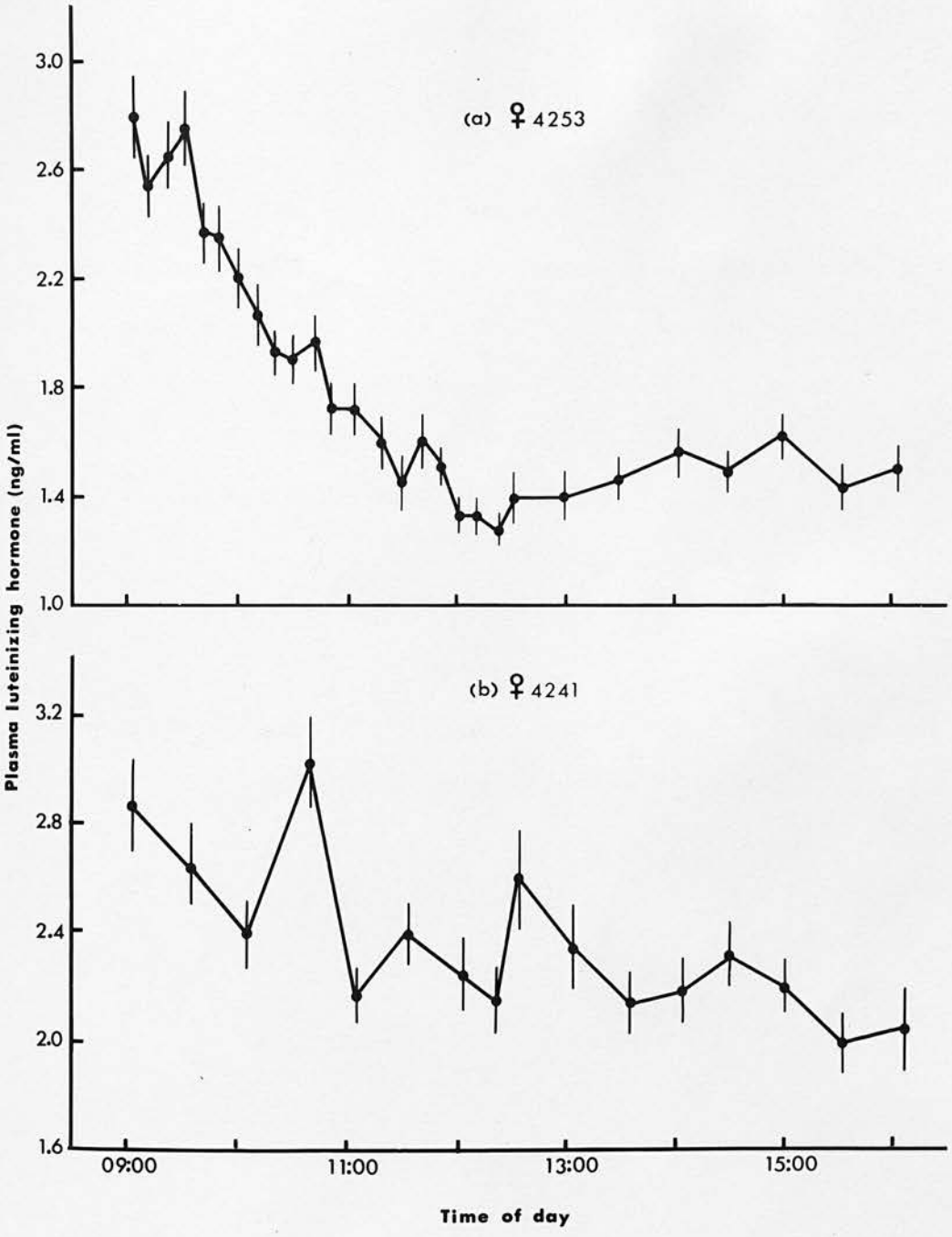
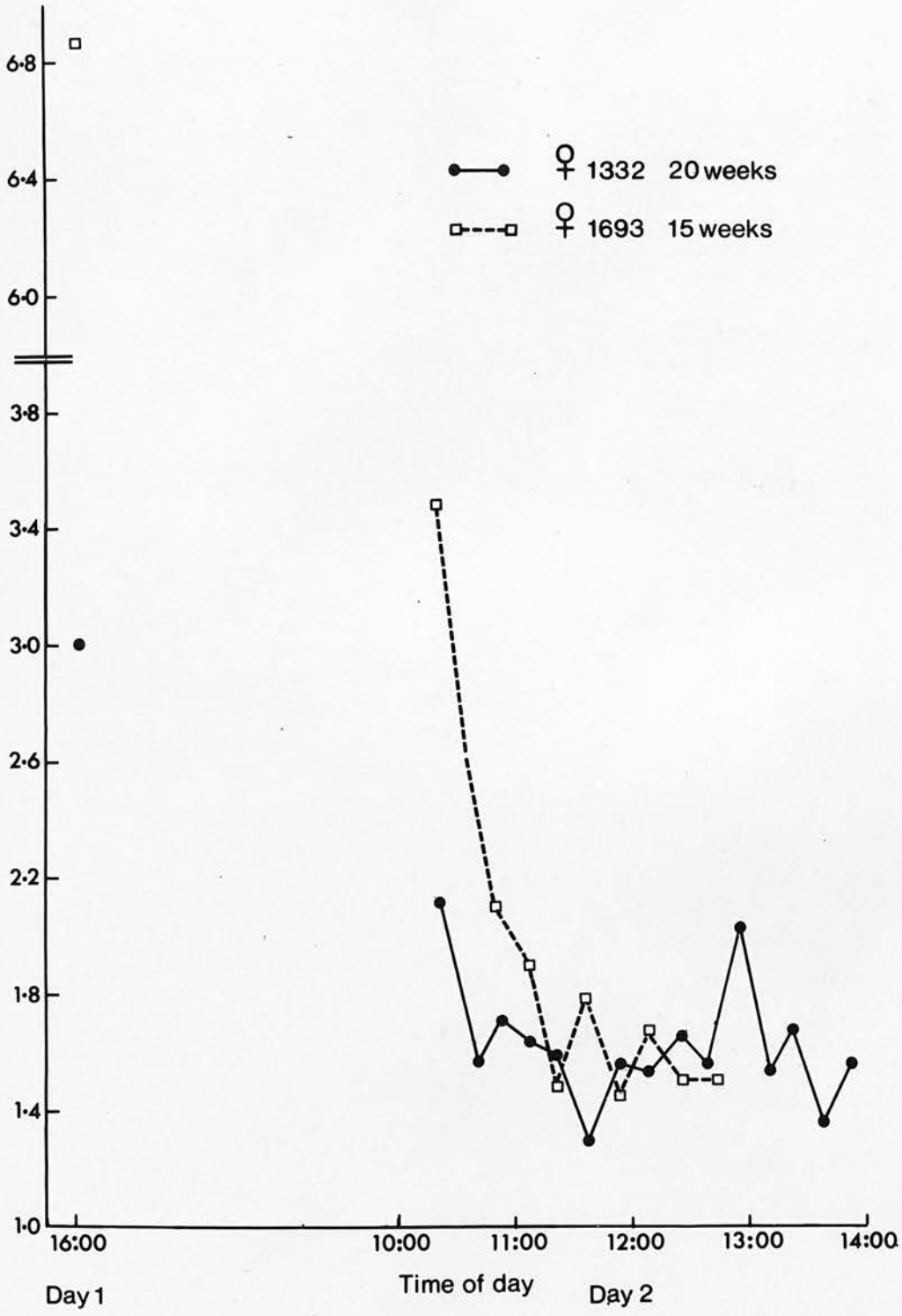

(a) ♀ 4253
(b) ♀ 4241
Plasma luteinizing hormone (ng/ml)
3.0
2.6
2.2
1.8
1.4
1.0
3.2
2.8
2.4
2.0
1.6
09:00
11:00
13:00
15:00
Time of day

Fig. 6


♀ 1332 20 weeks
♀ 1693 15 weeks
6·8
6·4
6·0
3·8
3·4
3·0
2·6
2·2
1·8
1·4
1·0
16:00
10:00
11:00
12:00
13:00
14:00
Day 1
Time of day
Day 2

LH peak was included in the sampling period (Fig. 5a).

D. IMMATURE HENS

Blood samples were taken at 10 to 15 min intervals for 3 h from one 15-week-old and one 20-week-old Thornber pullet (Fig. 6). During the initial sampling period, plasma LH levels fell from 6.9 to 1.5 ng/ml and from 3.0 to 1.6 ng/ml respectively. Thereafter levels remained depressed, though small fluctuations were evident similar in magnitude to those found in the laying hen.

Conclusion

It is concluded that in both intact and gonadectomized fowl, LH is not secreted from the pituitary at a constant rate, but as abrupt discharges. It appears that a single estimate for plasma LH concentration in individual cockerels and gonadectomized fowl may not be representative of their mean LH values.

E. ESTIMATION OF THE HALF-LIFE OF LH IN THE CIRCULATION

The differences in the pattern of episodic secretion between intact cockerels and gonadectomized fowl could possibly have been explained by a shorter half-life of LH in the blood of gonadectomized fowl.

This was investigated by measuring the rate of disappearance of ^{125}I-labelled LH from the circulation of two gonadectomized fowl (1 male and 1 female Thornber) and two intact cockerels (Thornbers). One wing vein of each bird was cannulated using the method described on page 32. 50 µl of a labelled LH preparation (AE1) were injected directly into the contralateral wing vein using a 100 µl Hamilton gas-tight micro-syringe. After allowing 2 minutes for the labelled LH to

become evenly distributed in the circulation, blood samples, each of 0.5 ml, were withdrawn through the cannulated wing vein at intervals of 5 min for the first 20 min, and then at 10 min intervals for a further 1 h 10 min.

The plasma (100 µl in 100 µl diluent) was incubated with 50 µl of anti-CM2 15/8 at a concentration of 1 : 200 for 24 h at 4° C. The antigen-antibody complex was precipitated by adding 50 µl of Donkey-Rabbit Precipitating Serum at a concentration of 1 : 4, and incubating for a further 24 h at 4° C. The assay tubes were then spun at 2500 r.p.m. for 40 min at 4° C; the supernatants were removed and the precipitates were counted on a Gamma Counter for 100 sec. The counts/100 sec were plotted against time on semilogarithmic paper and a straight line was fitted in each case by the method of least squares. The half-lives of LH were expressed as a function of the gradient of the line (Fig. 7).

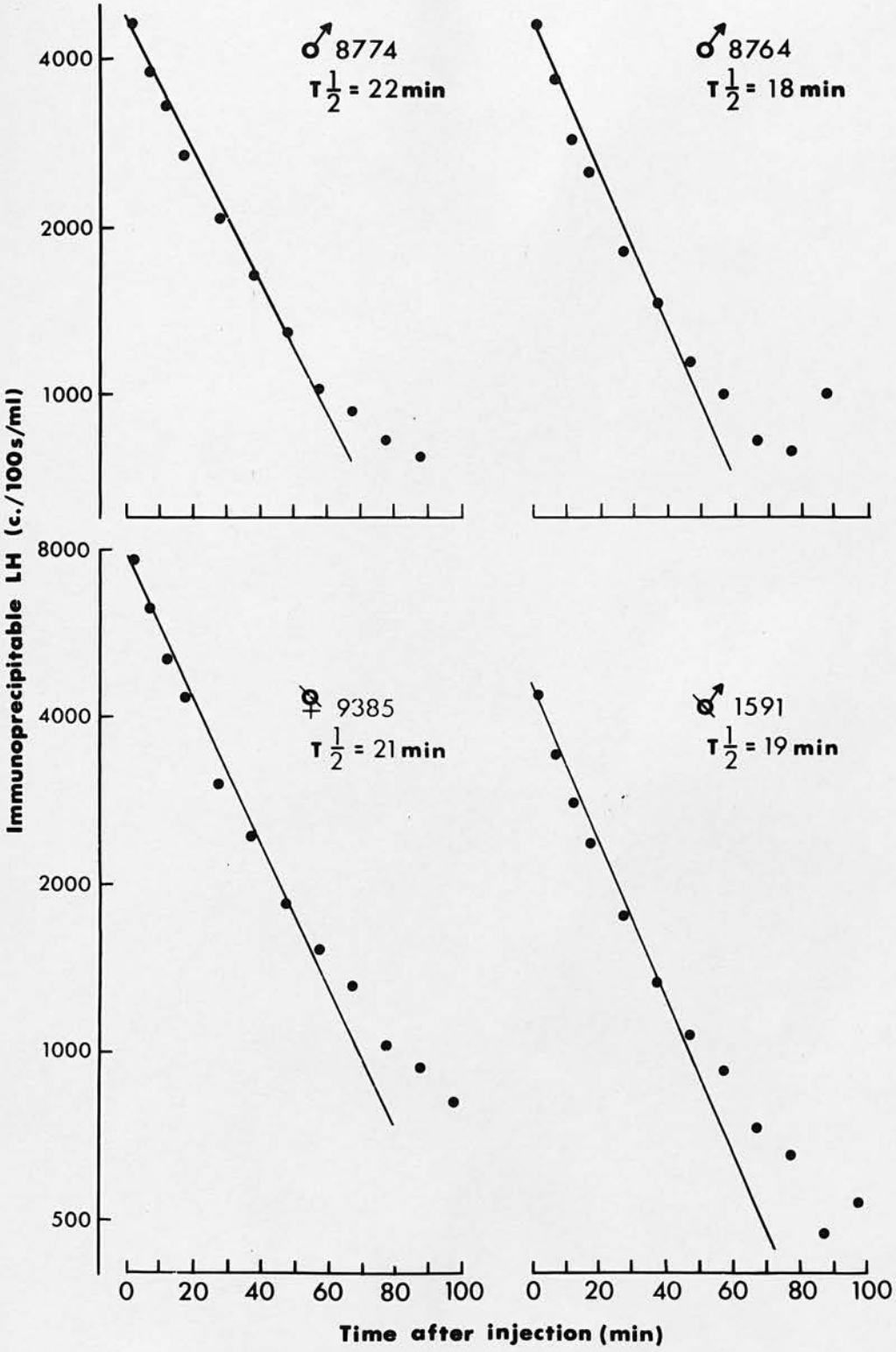
It was noticeable that the disappearance rate of LH in the circulation after either injection of ^{125}I-labelled LH or an episodic discharge could not be expressed by a single linear exponential, and hence all estimates of half-life were calculated from the initial phase of decay. The values obtained ranged from 18 to 22 min and there was no apparent difference in the rate of decay of LH between cockerels and gonadectomized fowl. The half-lives of LH for cockerels 8924, 8789 and 8431 (Fig. 2a, b, c) were estimated by the same method, using LH values on the declining slope of an episodic discharge, to be 22, 18 and 25 min respectively.

Since the half-life of LH in intact cockerels and gonadectomized fowl was similar, it is concluded that a difference in the pattern of

Figure 7

The rate of disappearance of exogenous (^{125}I-labelled) LH from the circulation of two intact cockerels (8774, 8764), one castrated cockerel (1591) and one ovariectomized hen (9385). Half-lives for LH are estimated by fitting a straight line, by the method of least squares, to the early phase of ^{125}I-labelled LH decay.

Fig. 7


♂ 8774
T½ = 22 min
♂ 8764
T½ = 18 min
♀ 9385
T½ = 21 min
♂ 1591
T½ = 19 min
Immunoprecipitable LH (c./100s/ml)
Time after injection (min)
4000
2000
1000
8000
4000
2000
1000
500
0
20
40
60
80
100

episodic secretion was not due to a different rate of LH decay.

II. REGULATION OF LH SECRETION IN THE INTACT HEN

A. LH SECRETION DURING THE OVULATORY CYCLE

Plasma LH levels were measured during complete ovulatory cycles in individual hens to confirm the observations made by Furr, Bonney, England & Cunningham (1973) of a single pre-ovulatory LH leak, to relate its occurrence to the photoperiod, and to determine if any other changes in plasma LH levels consistently occur at other times during the cycle.

The concentration of plasma LH was measured in blood samples, each of 0.5 ml, which were taken by venepuncture from 23 regularly laying hens (13 Thornbers, 10 Shavers) at 1.5 h intervals for 24 h (09:00 h to 09:00 h). Each sample was assayed in duplicate at 3 dilutions. Where an oviposition occurred within 36 h of the last blood sample being taken, this was used as evidence of an ovulation having occurred during or shortly after the 24 h study period. Eleven hens were thereby assumed to have ovulated during this period, and in each case one major peak of LH was observed. The LH changes during the ovulatory cycles of these hens are shown in Fig. 8. In the 12 hens which did not ovulate, no such LH peaks were observed (Fig. 9b). The LH changes in 2 of these hens are shown in Fig. 8 (hens 9407 and 8786).

During a pre-ovulatory surge LH levels rose to maxima of 200 to 300 % above mean baseline levels of 1.82 $\pm$ 0.08 (S.E.M.) ng/ml to 4.17 $\pm$ 0.22 ng/ml (range 3.00 to 4.54 ng/ml) and took between 4 and 7 h (mean 5.8 $\pm$ 0.34 h) to rise from and return to baseline levels. In all

but one case (Fig. 8, hen 328) there was a slight decrease in LH concentration immediately preceding the start of the LH rise. Fig. 9 compares the mean LH changes in 11 hens in relation to the time of ovulation, with the LH changes in the 13 hens which did not ovulate.

It was observed generally that the occurrence of an LH peak was closely linked to the dark period. The LH peaks preceding the first ovulation of a sequence in 6 hens all commenced between 0 and 3 h after the onset of the dark period, and highest LH values were measured at 00:30 h to 02:30 h (mean 01:30 h $\pm$ 20 min (S.E.M.)). All peaks preceding the terminal ovulation of a sequence in 4 hens commenced before the lights came on in the morning, though high LH values were observed as late as 13:00 h (Fig. 8, hens 571 and 572). Although no cases were observed of LH peaks commencing after the lights had come on, it can be predicted from examination of oviposition records that in hen 572 (Fig. 8) an LH peak preceding a terminal ovulation of a sequence must have occurred within 6 h of the last sample having been taken, i.e. LH levels began to rise later than 2 h after the lights had come on.

In 16 hens, a small though significant rise in LH concentration was observed at the onset of the dark period, irrespective of whether there was an ovulation later that day, and in 9 cases this took the form of a small peak. Examples are shown in Fig. 8 (hens 572, 8297, 222, 9407, 8786). In hens 8804, 9579 and 459 (Fig. 8) this rise may have been obscured by an early pre-ovulatory LH peak. Of the 23 hens studied, only 1 failed to show an elevation of LH at that time.

No other consistent changes in circulating LH levels were detected, although 5 of the 15 ovipositions which occurred during the

Figure 8

Variations in the concentration of plasma LH in individual hens during a 24-h period. All hens except nos 9407 and 8786 ovulated during or shortly after the 24-h sampling period. Vertical lines represent 95 % confidence limits. Black horizontal bars represent the hours of darkness.
OP, oviposition; C n/n , the number of the ovulation resulting from each LH peak and the number of eggs in that sequence.

Figure 9

(a) Mean changes in plasma LH concentration in eleven hens in relation to the time of predicted ovulation.

(b) Mean changes in plasma LH concentration throughout a 24-h period in twelve hens which did not ovulate.

Vertical lines represent $\pm$ S.E.M. Black horizontal bars represent the hours of darkness.

Fig. 8

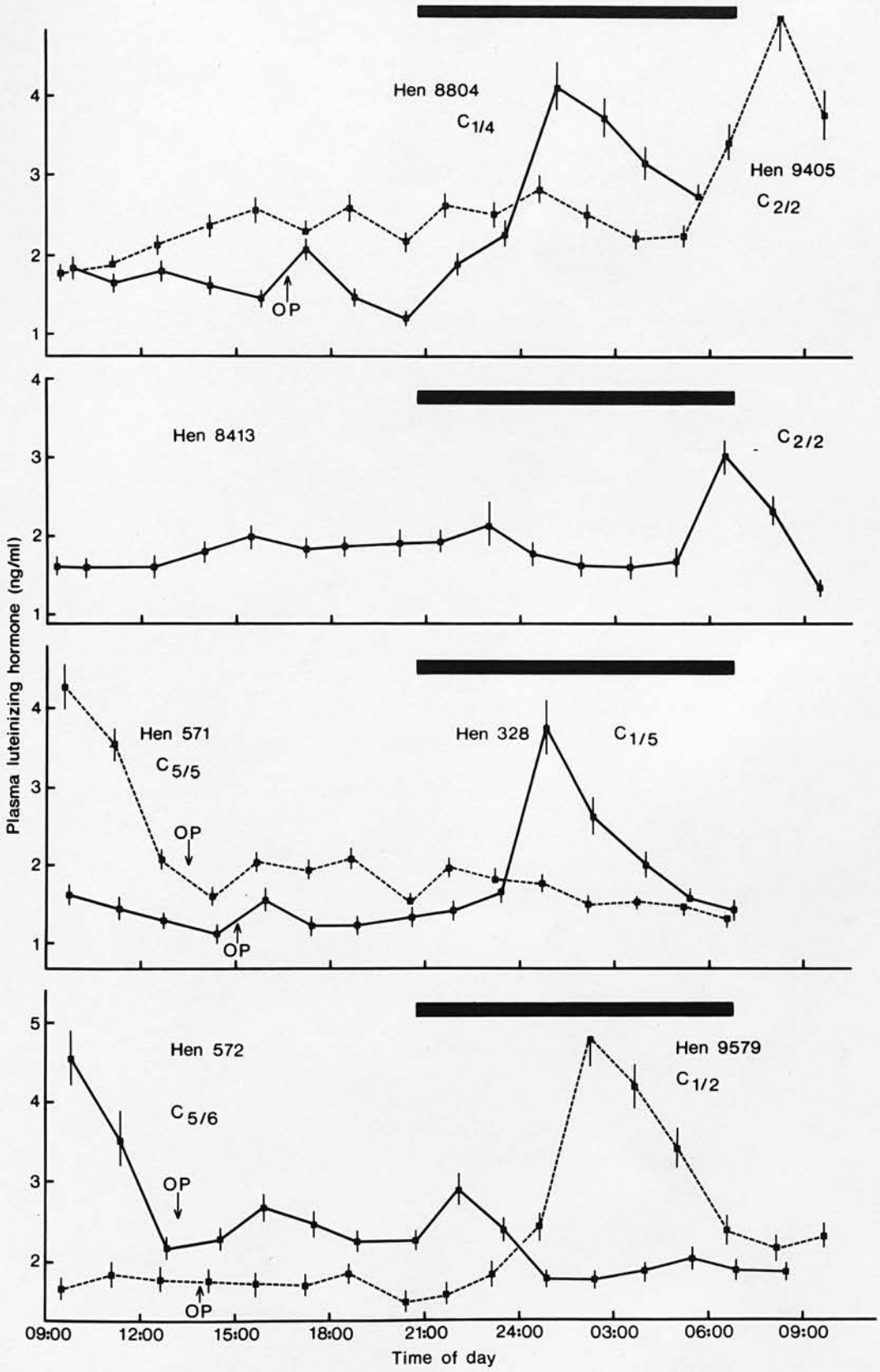

Hen 8804
C1/4
Hen 9405
C2/2
OP
Hen 8413
C2/2
Hen 571
C5/5
OP
OP
Hen 328
C1/5
Hen 572
C5/6
OP
OP
Hen 9579
C1/2
Plasma luteinizing hormone (ng/ml)
09:00
12:00
15:00
18:00
21:00
24:00
03:00
06:00
09:00
Time of day

Fig. 8 (cont.)

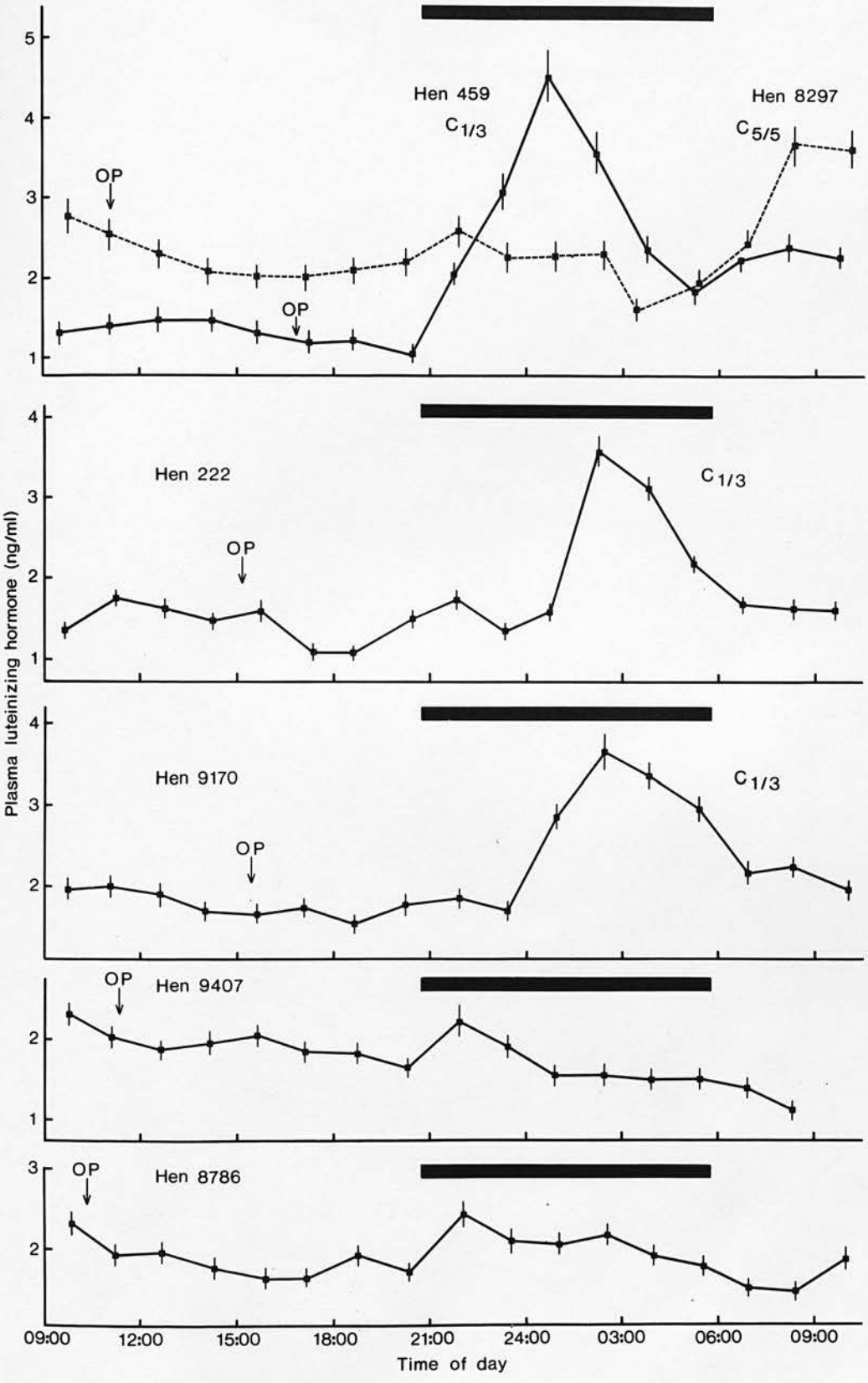

Hen 459
C1/3
Hen 8297
C5/5
OP
OP
Hen 222
C1/3
OP
Hen 9170
C1/3
OP
OP
Hen 9407
OP
Hen 8786
Plasma luteinizing hormone (ng/ml)
09:00
12:00
15:00
18:00
21:00
24:00
03:00
06:00
09:00
Time of day

Fig.9

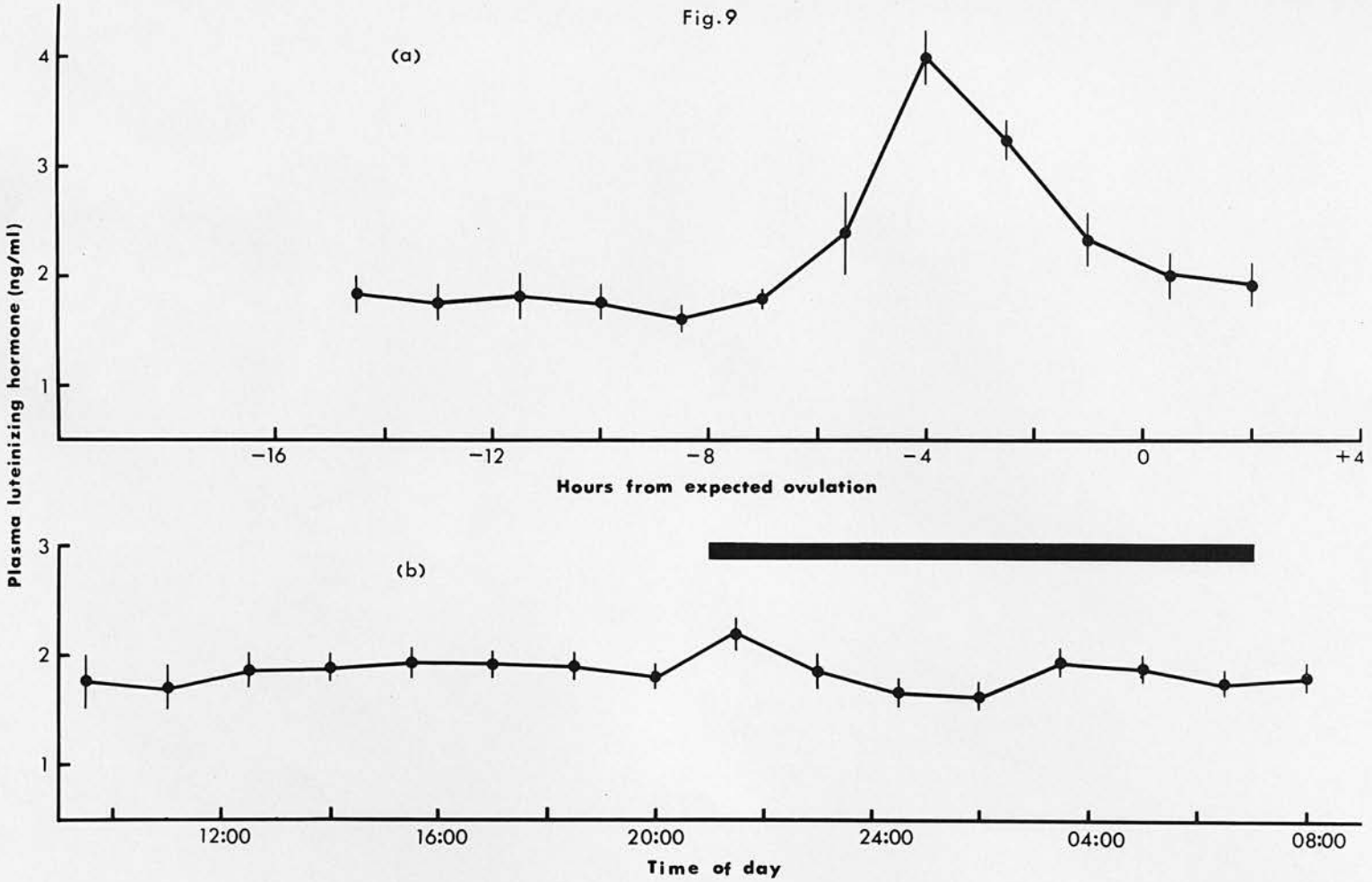

(a)
4
3
2
1
Plasma luteinizing hormone (ng/ml)
−16
−12
−8
−4
0
+4
Hours from expected ovulation
(b)
3
2
1
12:00
16:00
20:00
24:00
04:00
08:00
Time of day

Table 1. The time relationship between the pre-ovulatory LH peak and oviposition.

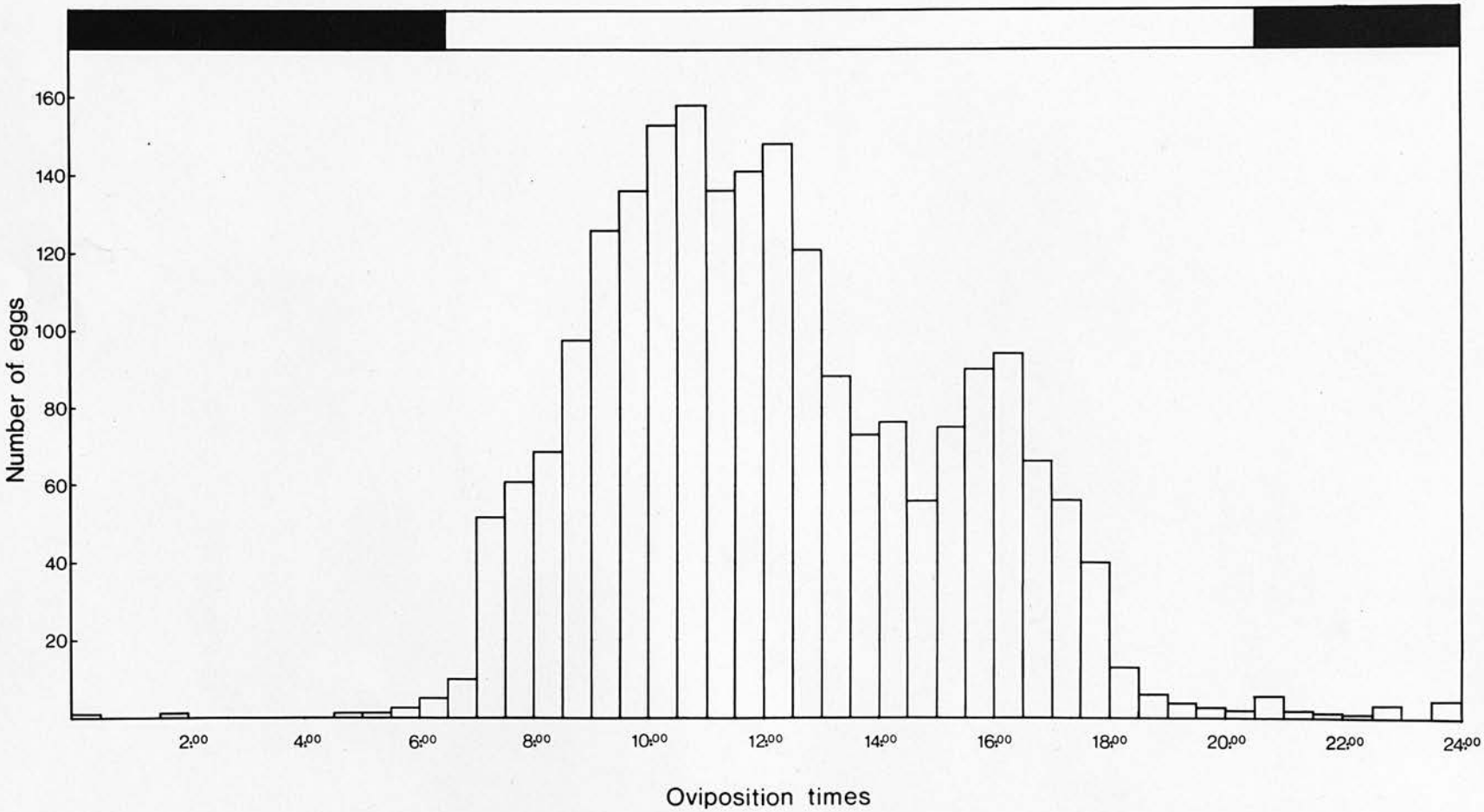
Hen no.	Ovulation associated with LH peak (see Fig. 8)	Maximum LH peak (time of day)	Oviposition of egg ovulated by LH peak (time of day)	Interval (h)* between LH peak and resulting oviposition	Interval (h)* between LH peak and oviposition of previously ovulated egg
459 (Shaver)	C 1/3	00:30	09:49	33.5	No egg laid
8804 (Thornber)	C 1/4	01:00	11:00	34.0	No egg laid
328 (Shaver)	C 1/5	01:00	08:13	31.5	No egg laid
222 (Shaver)	C 1/3	01:30	09:21	32.0	No egg laid
9579 (Thornber)	C 1/2	02:00	09:14	31.5	No egg laid
9170 (Thornber)	C 1/3	02:30	10:51	32.5	No egg laid
8413 (Thornber)	C 2/2	06:30	15:43	33.0	5.0
9405 (Thornber)	C 2/2	08:00	16:57	33.0	4.0
8297 (Thornber)	C 5/5	09:00	18:00	33.0	4.0
571 (Shaver)	C 5/5	09:30	18:08	32.5	4.0
572 (Shaver)	C 5/6	10:00	17:32	31.5	4.5

* to the nearest 0.5 h.

Figure 10

The distribution throughout the day of oviposition times of eggs laid by 132 hens during 16 days. Black horizontal bars represent the hours of darkness.

Fig.10


160
140
120
100
80
60
40
20
Number of eggs
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
Oviposition times

24 h study period were associated with a small rise in LH levels (e.g. Fig. 8, hens 8804, 328). No differences were observed between Shaver and Thornber hens in the characteristics of their pre-ovulatory LH surges.

In 5 hens with an LH peak, the interval between highest LH values and the oviposition of the previously ovulated egg was 4.3 ± 0.20 (S.E.M.) h (Table 1). If ovulation occurred within 14 to 75 min of oviposition (Warren & Scott, 1935), then it is estimated that ovulation occurred about 4 to 5 h after peak LH values.

As shown in Table 1, a fairly constant time relationship of 31.5 to 34.0 h (mean 32.5 ± 0.25 (S.E.M.) h) could be established between maximum LH levels and the resulting oviposition. When the distribution of eggs laid during 16 days by 132 Shavers of the same age and maintained under the same conditions were plotted according to distribution throughout the day, it is seen that most eggs were laid during the hours of light, between 07:00 h and 18:00 h, and very few were laid during the hours of darkness (Fig. 10). It appears from comparison of these results that this pattern of oviposition times is dependent on the close relationship between the photoperiod and the peak of LH occurring 31.5 to 34.0 h before each oviposition.

B. ROLE OF GONADAL STEROIDS IN THE REGULATION OF CYCLIC LH SECRETION

It is known that concentrations of circulating androgens, oestrogens and progesterone are highest between 9 and 4 h before ovulation, and that rising levels shortly precede or accompany the pre-ovulatory rise in LH secretion (see page 23). The possible role of these steroids in initiating the pre-ovulatory LH peak was

examined using two different approaches. The first was an attempt to neutralize the biological activity of each of these steroids by passive immunization with specific antisera and examine the effect of the treatment on the occurrence of the pre-ovulatory LH peak. The second involved injection of the steroids at various times during the ovulatory cycle to see if they would induce LH release.

1. Effects of injections of antisera to gonadal steroids on the pre-ovulatory LH peak

Antisera to oestradiol-17β, progesterone and testosterone were generously provided by Dr R.J.Scaramuzzi (M.R.C. Unit of Reproductive Biology). The pooled antiserum to oestradiol-17β was raised in ovariectomized sheep against oestradiol-17β conjugated to bovine serum albumin in the 6 position (Scaramuzzi, Corker, Young & Baird, 1974) and blocked oestrous behaviour in progesterone-primed, oestradiol-injected ovariectomized sheep (Scaramuzzi, 1975). The antisera to progesterone and testosterone were raised in intact sheep. The antiserum to progesterone was from a single bleed (91870/13) and in a final volume of 300 µl at a dilution 1:10,000 bound 16 pg of progesterone. The antiserum to testosterone bound 15 pg of testosterone at a dilution of 1:10,000 (final vol. 300 µl). Each antiserum showed no major cross reaction with steroids other than the one against which it was raised (R.J. Scaramuzzi, personal communication). Normal sheep serum was used for control injections.

The Shaver hens selected for this experiment were all laying regular sequences of 3 or more eggs and the approximate times of ovipositions could be predicted from an examination of oviposition

records of previous sequences of each hen. The expected time of the LH peak could be then estimated, since the interval between peak LH levels and the oviposition of the egg resulting from that peak is constant (Table 1).

Undiluted antisera or normal sheep serum were injected intravenously at a dose of 1.25 ml/kg into laying hens which were due to ovulate an early egg of a sequence. Each hen was injected, using one of the following treatments:-

Treatment i

The antisera or control serum were injected on a single occasion during the afternoon between 17.0 h and 11.5 h before the predicted time of ovulation. Plasma LH levels were measured in blood samples taken at the time of the injection and at 1 hourly intervals for 12 h from the onset of darkness, i.e. from just before the time when the pre-ovulatory LH surge associated with the first ovulation (C_1) of a sequence is normally initiated (see page 50).

Treatment ii

In order to examine the effects of further raising the titre of the progesterone antiserum in the circulation, hens were each given 2 injections of 1.25 ml progesterone antiserum/kg separated by a period of 3 h. The injections were given between 18.5 h and 12.0 h before the predicted time of an ovulation. Blood samples were taken as in treatment i. Control hens were given a similarly timed double dose of normal sheep serum.

Treatment iii

1.25 ml oestradiol-17β antiserum/kg or normal sheep serum were injected during the afternoon on 2 successive days to investigate the

effects of a longer-term neutralization of the biological effects of oestrogen on the initiation of the pre-ovulatory surge of LH. Plasma LH levels were measured in samples taken immediately before each injection and at twelve 1 h intervals during the period of darkness following the second injection.

There was no evidence to suggest that the antisera or normal sheep serum affected the basal secretion of LH except after treatment ii where 2 injections of either progesterone antiserum or normal sheep serum depressed plasma LH levels at the onset of darkness by means of 0.44 ± 0.04 ng/ml (n = 2) and 0.32 ± 0.08 ng/ml (n = 4) respectively. It was therefore likely that this slight depression in the basal secretion of LH was due to adverse effects of the large volume of sheep serum injected.

Variations in plasma LH levels during the time when pre-ovulatory LH peaks were predicted in hens injected with steroid antisera or with normal sheep serum are shown in Fig. 11a - d.

a. Testosterone antiserum

Four hens were injected with testosterone antiserum between 14.5 and 11.5 h before the predicted time of ovulation (treatment i). In one hen (1823, Fig. 11a) no eggs were laid for 4 days after the injection, and it is assumed that ovulation had been blocked. This hen had what seemed to be a pre-ovulatory LH peak during which LH levels rose to maxima of 146 % above baseline levels (taken as the level of LH in the first serial sample at the onset of darkness). However, this was less than the corresponding mean rise of 209 ± 29.5 (S.E.M.) % (n = 5) in the controls (Fig. 11b i).

In 2 of the 4 hens (1695, 2039; Fig. 11a), LH levels rose by 242 % and 192 %. In both cases, the oviposition of the egg resulting from that LH peak occurred 2 or 4 h later than predicted, and in hen 2039 (Fig. 11a) the delayed oviposition terminated the sequence since no egg was laid on the following day. However, as pre-ovulatory LH levels started to rise at the predicted time and the interval between peak LH values and oviposition (33.0 and 34.75 h) was greater than in either of the 5 hens injected at similar times with normal sheep serum (mean 31.5 $\pm$ 0.44 (S.E.M.) h) or in all other groups of antiserum-injected hens (mean 30.7 $\pm$ 0.26 h) (Table 2), it is deduced that the effect of testosterone antiserum was not to delay the pre-ovulatory LH peak but to either delay the ovulation resulting from that peak or delay oviposition by affecting oviduct motility.

In the remaining hen (1758), there was no apparent effect on the pre-ovulatory LH peak or on the timing of oviposition of the egg resulting from that peak. However, no eggs were laid on the 2 days following that oviposition and it is possible that the testosterone antiserum blocked ovulations occurring more than 24 h after its injection.

b. Oestradiol-17β antiserum

In the 5 hens injected with antiserum to oestradiol-17β between 17.0 and 12.0 h before the predicted time of ovulation, pre-ovulatory LH levels rose by a mean of 242 $\pm$ 44 (S.E.M.) % above base-line values (Fig. 11c i) compared with a rise in the 5 controls of 209 $\pm$ 29.5 % (Fig. 11b i). The large rises of 406 % and 259 % in hens 4154 and 4133 respectively (Fig. 11c i) may be associated with the relatively long sequence lengths in these birds (8 to 10 eggs, and 12

to 19 eggs respectively) rather than to any effects of the oestradiol-17β antiserum. The time of oviposition of eggs resulting from the LH peaks was not effected in any of the 5 hens.

Three hens were injected with oestradiol-17β antiserum on 2 successive days (Fig. 11c iii) and, in each case, the predicted pre-ovulatory LH peak after the second injection was significantly smaller ($P < 0.05$) than in the 4 control hens injected at the same time with normal sheep serum; mean maximal increases in LH levels were 99 ± 18.5 (S.E.M.) % compared with 154 ± 19.8 % in the controls (Fig. 11b iii). However, in each case, this small rise in LH appears to have been sufficient to induce an ovulation. Ovipositions of the eggs resulting from these small LH peaks occurred 2.5 or 3.0 h later than predicted (Table 2) as did subsequent eggs of each sequence. This appears to have resulted from a delay in the occurrence of the predicted LH peak in each hen. In 2 of the 3 hens, oviposition of the egg ovulated after the first injection of antiserum was also delayed by 1.5 h (Table 2). However, since this egg was in the oviduct at the time of the second injection it is probable that the delaying effect was a result of this injection.

How did you do these stats?

c. Progesterone antiserum

In the 6 hens given a single injection of progesterone antiserum between 17 and 12 h before a predicted ovulation (Fig. 11d i), pre-ovulatory LH levels rose by a mean maximum of 193 ± 19.8 (S.E.M.) % which was similar to that (209 ± 29.5 %) in the 5 control hens given a single injection of normal sheep serum (Fig. 11b i).

However, in 3 of these hens (4158, 4160, 4217) oviposition of the eggs resulting from the LH peaks appeared to be delayed by between

2 and 4 h (mean 2.7 ± 0.7 h) (Table 2). In hens 4160 and 4217, subsequent ovipositions of that sequence were also delayed, and in hen 4158 the delayed oviposition became the last of the sequence, since no egg was laid on the following day. As the interval between pre-ovulatory LH values and oviposition (31.1 ± 0.6 h) was similar to that in the 5 control hens (31.5 ± 0.44 h), it appears that the LH peak was delayed by between 2 and 4 h (Fig. 1d i; Table 2). In hen 4217, the oviposition of the egg in the oviduct at the time of injection was also delayed by 2 h (Table 2). In the remaining 3 hens (4135, 4190, 9425) LH peaks were observed at the expected times and the times of oviposition appeared to be unaffected (Fig. 1d i; Table 2).

In 1 of the 2 hens injected with progesterone antiserum twice in the same day, i.e. 15.5 and 12.25 h before ovulation (treatment ii; Fig. 11d ii, hen 1913), the timing of the predicted LH peak was unaffected, though the magnitude of the peak was reduced compared with that of the 2 hens injected with normal sheep serum at the same times (86 % rise versus 158 % and 92 % rise above base-line levels) (cf. Figs. 11d ii and 11b ii). In the other hen, only part of the pre-ovulatory LH peak was included during the sampling period. However, it could be seen that LH levels were rising more gradually than in the control hens, and the time of oviposition of both the egg resulting from that LH peak and the egg in the oviduct at the time of the injection was delayed by 2 h (Table 2).

Conclusion

It was concluded that although none of the treatments with antisera raised against gonadal steroids completely prevented the occurrence of the pre-ovulatory peak of LH, the injection of antiserum

Figure 11

Variations in the concentration of plasma LH around the time of predicted pre-ovulatory peaks in hens injected with antisera to gonadal steroids and in 8 of 11 hens injected with normal sheep serum. 95 % confidence limits were estimated for each LH value, but are only shown in Fig. 11a. The number of each hen is shown alongside the LH peak. C n represents the number in a sequence of the ovulation resulting from each LH peak. The small Roman numerals refer to the different injection schedules which are described in the text (page 53). The black horizontal bars represent the hours of darkness.

Fig.11

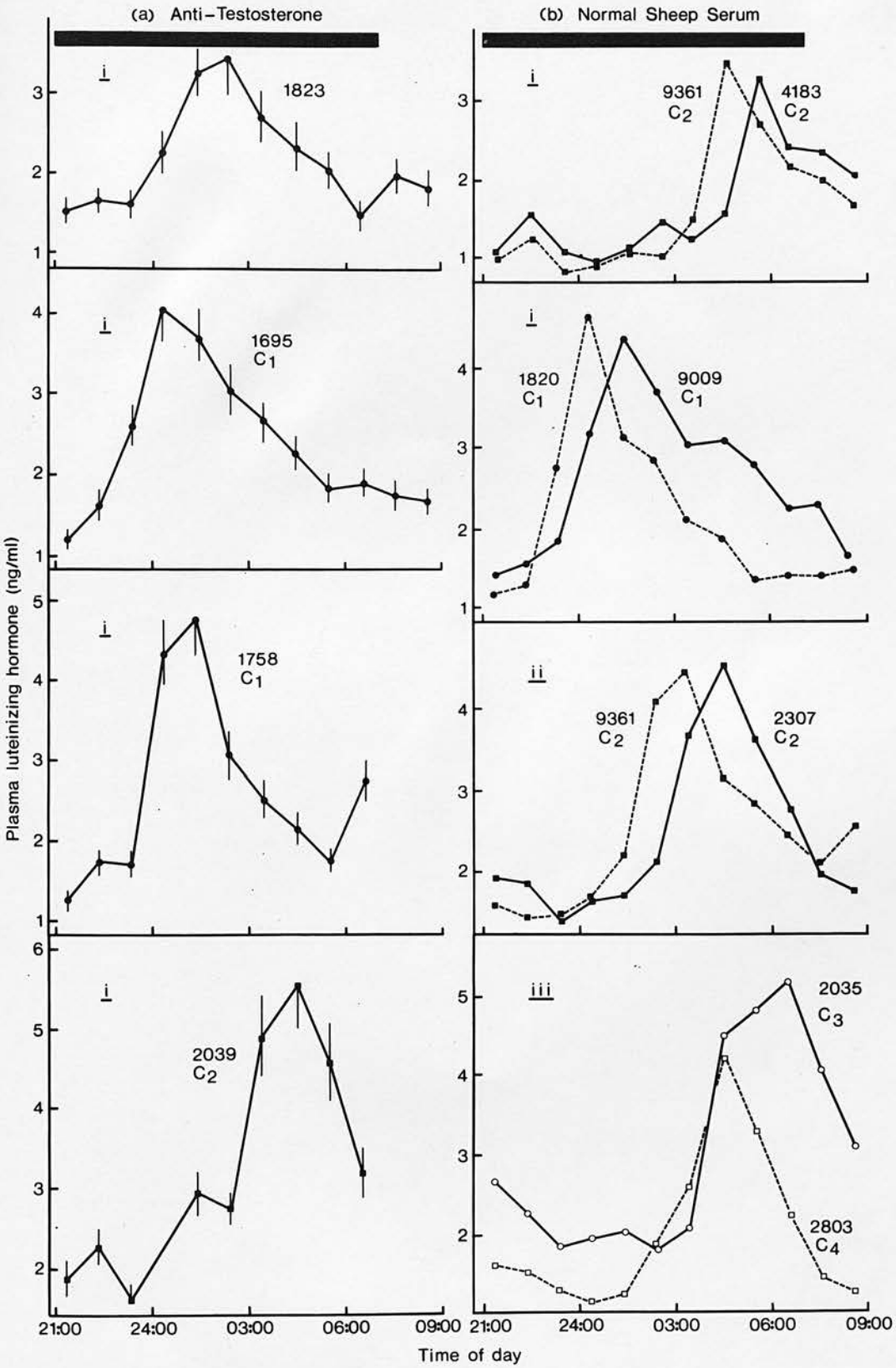

(a) Anti-Testosterone
(b) Normal Sheep Serum
i
1823
9361
C2
4183
C2
1695
C1
1820
C1
9009
C1
1758
C1
ii
9361
C2
2307
C2
2039
C2
iii
2035
C3
2803
C4
Plasma luteinizing hormone (ng/ml)
21:00
24:00
03:00
06:00
09:00
Time of day

Fig. 11 (cont.)

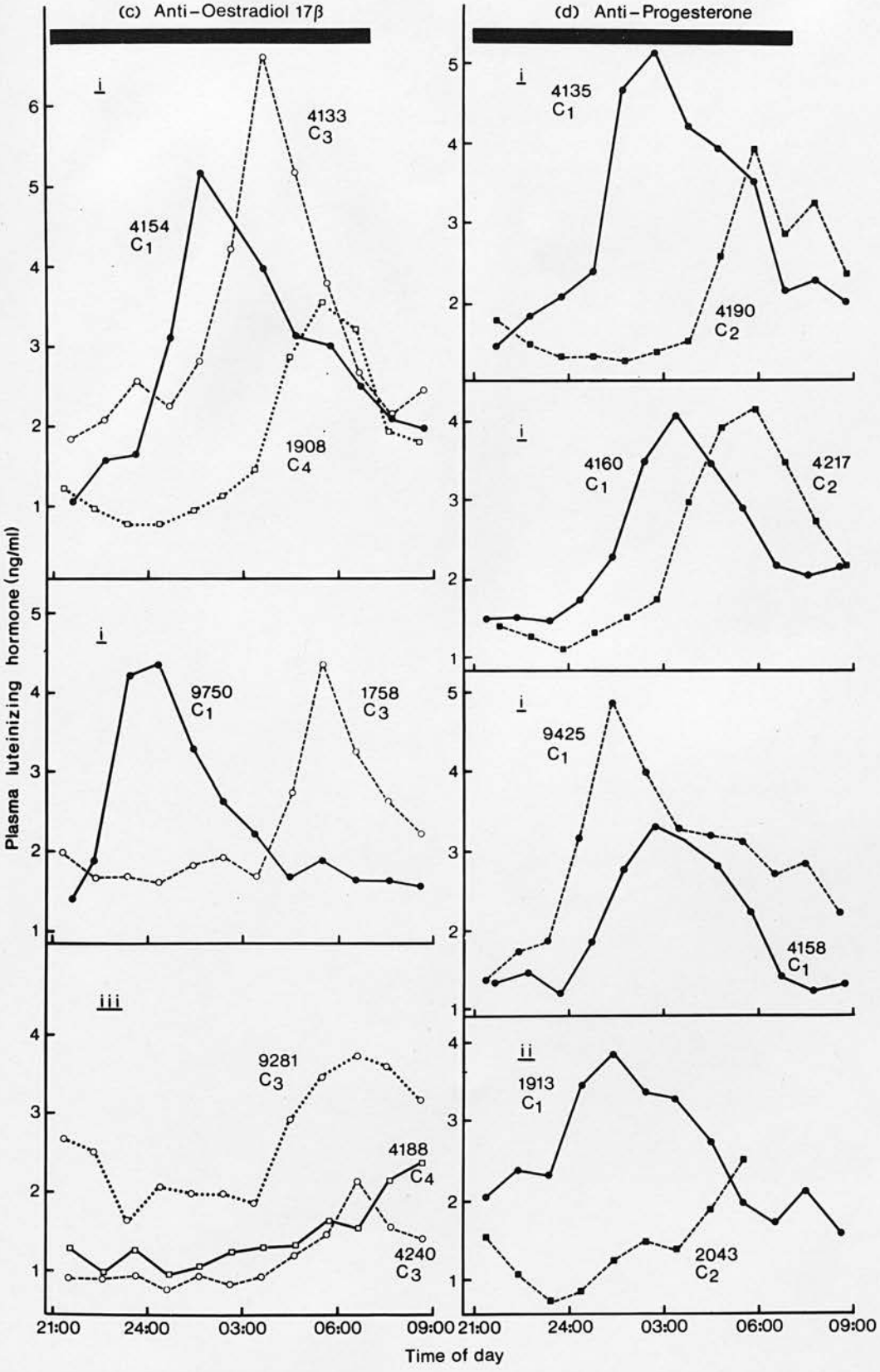

(c) Anti-Oestradiol 17β
(d) Anti-Progesterone
i
4133
C3
4154
C1
1908
C4
i
9750
C1
1758
C3
iii
9281
C3
4188
C4
4240
C3
i
4135
C1
4190
C2
i
4160
C1
4217
C2
i
9425
C1
4158
C1
ii
1913
C1
2043
C2
Plasma luteinizing hormone (ng/ml)
21:00
24:00
03:00
06:00
09:00
Time of day

Table 2. The effect of injections of antisera to gonadal steroids on ovulation and oviposition

Hen no.	Number of eggs in sequence	Time of injection in relation to ovulation	*Delay (+) in ovulation (h)	Interval between peak LH levels and resulting oviposition (h)
Treatment i. Single i.v. injection of 1.25 ml antiserum/kg.				
a. Testosterone antiserum				
1823	2 - 4	11.50 h before C_1	Blocked or delayed for 3 days	-
1695	4 - 5	11.50 h before C_1	+ 2.0	33.00
1758	3	11.50 h before C_1	No effect	32.25
2039	4	14.50 h before C_2	+ 4.0	34.75
b. Normal sheep serum				
9009	4 - 6	12.50 h before C_1	No effect	31.75
1820	3 - 6	12.00 h before C_1	No effect	31.50
9361	3	16.50 h before C_1	No effect	31.50
4183	3	17.75 h before C_2	+ 2.5	32.75
1831	5 - 8	15.75 h before C_3	No effect	30.00
c. Oestradiol-17β antiserum				
9750	3 - 5	11.75 h before C_1	No effect	31.00
4154	8 - 10	12.75 h before C_1	No effect	30.00
4133	12 - 19	15.50 h before C_3	No effect	28.50
1758	3 - 5	17.00 h before C_3	No effect	31.00
1908	7	16.75 h before C_4	No effect	29.50
d. Progesterone antiserum				
4135	5 - 10	12.25 h before C_1	No effect	30.50
4158	3 - 5	12.50 h before C_1	+ 4.0	33.75
4160	3 - 4	12.75 h before C_1	+ 2.0	31.00
9425	3 - 4	12.75 h before C_1	No effect	31.75
4217	3 - 6	15.00 h before C_2	+ 2.0†	30.25
4190	7 - 9	17.00 h before C_2	No effect	29.25

Table 2 (contd.)

Hen no.	Number of eggs in sequence	Time of injection in relation to ovulation	*Delay (+) in ovulation (h)	Interval between peak LH levels and resulting oviposition (h)
Treatment ii. Two i.v. injections of 1.25 ml antiserum/kg on one day.				
b. Normal sheep serum				
9361	6 - 9	16.50 h 14.50 h before C_2	No effect	28.75
2307	4 - 8	17.75 h 15.50 h before C_2	No effect	29.75
d. Progesterone antiserum				
1913	6 - 12	15.50 h 12.25 h before C_1	No effect	30.25
2043	7 - 9	18.50 h 16.00 h before C_2	+ 2.0†	?
Treatment iii. Single i.v. injection of 1.25 ml antiserum/kg on two successive days.				
b. Normal sheep serum				
4220	3	11.50 h before C_2 20.00 h before C_3	No effect	32.00
2035	6 - 7	13.50 h before C_2 15.50 h before C_3	No effect	28.00
2010	7 - 8	13.75 h before C_2 15.50 h before C_3	No effect	30.50
2803	6 - 10	15.25 h before C_3 16.50 h before C_4	No effect	31.00
c. Oestradiol-17β antiserum				
4240	5	11.50 h before C2 19.00 h before C_3	+ 1.5 + 2.5	32.00
9281	5 - 6	15.50 h before C_2 17.50 h before C_3	+ 1.5 + 3.0	31.00
4188	6 - 7	12.50 h before C_3 19.00 h before C_4	No effect + 3.0	31.00

* , as estimated by comparison with oviposition/ovulation times for previous sequences.

† , oviposition of egg in oviduct at time of injection delayed by 2.5 h.

? , peak LH levels not included in sampling period.

to progesterone on a single day delayed and in some cases reduced the pre-ovulatory LH peak. Similar effects resulted from injection of the antiserum to oestrogen, when given on 2 successive days, while a single injection did not modify the LH peak. This suggests that both oestrogen and progesterone may be involved in the positive feedback mechanism. Testosterone, on the other hand, appeared to delay ovulation or oviposition without affecting the timing of the pre-ovulatory LH peak and may not therefore be necessary for the functioning of the positive feedback mechanism.

2. Induction of LH release by gonadal steroids

The second method by which the role of gonadal steroids in the control of cyclic LH secretion was examined involved the injection, at various stages of the ovulatory cycle, of each of the steroids which have been shown to increase in the blood at about the same time as the pre-ovulatory peak of LH (page 23), and determine which steroids would stimulate LH secretion. Additionally, the effects of the adrenal steroid, deoxycorticosterone, were examined since it has been found to induce ovulation (Fraps, 1955).

Summary of experiments

a. Progesterone

Preliminary investigations showed that the intramuscular injection of laying hens with 0.5 mg progesterone/kg, dissolved in a solution of propylene glycol, often resulted in an elevation of plasma LH levels in a blood sample taken 1.5 h later. Assuming that progesterone was exerting a positive feedback effect on LH secretion, tests were carried out to determine a suitable route of steroid administration, a suitable frequency of blood sampling and a suitable dose-level of progesterone.

(i) Route of steroid administration

In order to decide on the means of injecting progesterone, Shaver laying hens were given either an intraperitoneal, subcutaneous or intramuscular injection of 0.5 mg progesterone/kg. A blood sample of 0.75 ml was withdrawn by means of venepuncture immediately before the steroid injection and at 1.5 h intervals for a period of 4.5 h afterwards.

Intraperitoneal

In 4 hens injected intraperitoneally, the concentration of LH in the plasma rose steadily from a mean level of 1.66 ± 0.12 (S.E.M.) ng/ml to 3.09 ± 0.23 ng/ml over a period of 4.5 h. The true magnitude of the response could not be estimated since no further samples were

withdrawn after 4.5 h. However, it was apparent that the rise in LH secretion was more gradual than that in a natural pre-ovulatory LH surge (see Fig. 8) and consequently, for this reason the intraperitoneal route of injection was considered unsuitable for further experiments.

Subcutaneous

The subcutaneous injection of 4 hens in the nape of the neck resulted in a rise in plasma LH levels of only 0.89 ng/ml. The pre-injection concentration was 2.10 ± 0.31 ng/ml and it rose to mean levels of 3.00 ± 0.22 ng/ml at 1.5 h later. Thereafter, plasma LH levels declined to reach base-line values by 4.5 h after injection. It was noticeable that some of the progesterone solution escaped from the injection site, and since, as a consequence, an accurate dose could not be given, this was considered an unsuitable method of progesterone administration.

Intramuscular

The intramuscular route involved injection into the breast muscle. In the 4 hens given an intramuscular injection of progesterone, LH levels rose from a mean of 1.86 ± 0.27 (S.E.M.) ng/ml to reach maximal values of 4.05 ± 0.47 ng/ml at 1.5 h later. Thereafter, LH levels declined, but were still 0.64 ng/ml above pre-injection values after 4.5 h. Of the three routes of injection investigated, the intramuscular route was considered the most suitable and was selected for all further experiments, since it produced an LH response most similar to a naturally occurring LH peak.

(ii) Frequency of blood sampling

Two Thornber hens were cannulated (see page 32) and given a single intramuscular injection of 0.5 mg progesterone/kg at 4 to 5 h

before the terminal oviposition of a sequence. Blood samples were withdrawn through the cannulae at 15 to 30 min intervals for a period of 6 to 7 h. This frequency of sampling made it possible to determine precisely the magnitude and duration of the resulting LH surge. Maximal incremental changes in plasma LH levels of 2.58 and 2.05 ng/ml from pre-injection values of 1.53 and 1.98 ng/ml were reached after 50 and 100 minutes respectively and the duration of elevated LH levels in each case was 4.5 h. It was noticeable that LH levels rose to a peak much faster following the intramuscular injection of 0.5 mg progesterone/kg than during a naturally-occurring LH peak and the duration of elevated LH levels was comparatively reduced. However, the magnitude of the LH rise was similar to the pre-ovulatory LH surge. In order to follow the main characteristics of the steroid-induced LH surge in further experiments where the use of venepuncture restricted the number of samples that could be withdrawn (see page 34), it was decided to take 1 sample of 0.75 to 1 ml immediately prior to the injection followed by 4 more at 15 min intervals and thereafter up to 8 more at 30 min intervals.

(iii) Dose-level of progesterone

Eight laying Shavers were each injected intramuscularly at fortnightly intervals in the afternoon with 4 dose-levels of progesterone. Plasma LH levels were measured in blood samples withdrawn by venepuncture at the same time intervals after injection as described above. In all but 4 cases, plasma LH concentrations started to rise after a latent period of about 30 min and reached maximal levels within 90 to 120 min. The mean maximal incremental changes in LH levels after injections of 0.05, 0.1, 0.5 and 2.0 mg

progesterone/kg were 0.56 ± 0.13 (S.E.M.) ng/ml (n=8), 1.55 ± 0.36 ng/ml (n=7), 1.73 ± 0.25 ng/ml (n=7) and 1.59 ± 0.19 ng/ml (n=6) respectively. In subsequent experiments, the dose of 0.5 mg/kg was used since it gave the largest and the most uniform response.

It was noticeable that in 4 hens in which progesterone caused no rise in plasma LH concentration, the level of LH at the time of injection was slightly higher than in other hens. Each of these 4 hens had been injected shortly before an oviposition that was not the terminal one of a sequence, and it seemed likely that injections had been given just after a pre-ovulatory LH peak. The possibility that this lack of response was not due to variation among individual hens but due to variations in the response during the ovulatory cycle was investigated in more detail.

(iv) Effects of progesterone given at various stages of the ovulatory cycle on LH secretion.

Nine to sixteen-month old Shaver hens, laying regular sequences of 4 to 10 eggs were selected for this study. The stages of the ovulatory cycle at which progesterone was injected were defined in relation to the time of ovulation. This was taken to occur within 14 to 75 min of oviposition (Warren & Scott, 1935). The first ovulation of a sequence, which is not associated with a previous ovulation, was estimated to occur at approximately 05:00 h. This was based on the observations that the interval between the peak of the pre-ovulatory LH surge and ovulation is about 4 h (page 51) and that in hens maintained under the same lighting conditions as were used in this study, peak pre-ovulatory LH levels occur at about 01:30 h on the day of the first ovulation in a sequence (page 50). LH levels were

measured in blood samples (0.75 ml) taken by venepuncture at the time of progesterone injection and at 15 and 30 min intervals afterwards (page 61) for a further 3 to 5 h.

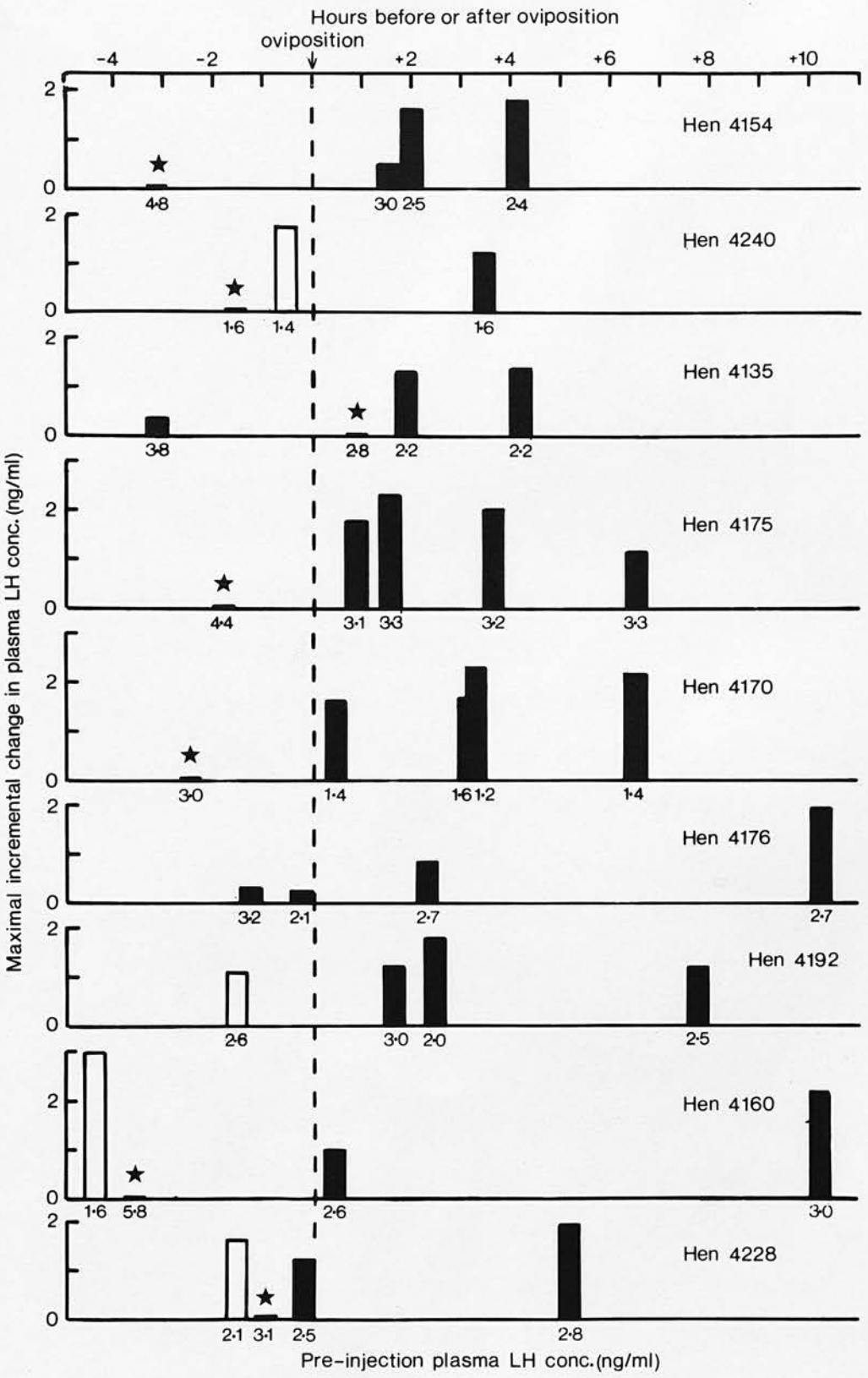
When 9 hens were injected on four or five fortnightly occasions with 0.5 mg progesterone/kg at various times between 4 h before and 10 h after an ovulation, there was considerable variation in the LH responses within each individual (Fig. 12). There was little or no response if the steroid was injected any time during the 4 h period before an oviposition that was accompanied by an ovulation. Under these circumstances pre-injection LH concentrations were high, because the blood samples had been taken on the declining slope of a pre-ovulatory LH surge. However, in cases where an oviposition was not accompanied by an ovulation, i.e. at the end of a sequence, there had been no immediately preceding LH surge and the pre-injection LH concentration was not high. In these circumstances the injection of progesterone during the 4 h period before an oviposition resulted in an induced LH surge (Fig. 12; hens 4240, 4192, 4160, 4228). These findings suggested that there was a change in the sensitivity of the hypothalamo-hypophysial complex to progesterone after exposure to a pre-ovulatory release of LH and/or ovarian steroids.

This possibility was studied more systematically by injecting a further 37 hens with 0.5 mg progesterone/kg 12 to 8 h, 8 to 4 h or 4 to 0 h before a predicted ovulation. These periods were expected to include the various phases of the spontaneous pre-ovulatory LH surge (see page 49). The resulting changes in LH secretion were compared with those observed after injecting the same hens a fortnight later more than 4 h after one ovulation and less than 12 h before the next

Figure 12

Maximal incremental changes in plasma LH concentration in nine laying hens following an intramuscular injection of 0.5 mg progesterone/kg at various times between 4 h before and 10 h after ovipositions which were either accompanied (black bars) or unaccompanied (white bars) by ovulations. ✱ , no resulting incremental change.

Fig.12


Hours before or after oviposition
oviposition
-4
-2
+2
+4
+6
+8
+10
Hen 4154
4·8
3·0 2·5
2·4
Hen 4240
1·6
1·4
1·6
Hen 4135
3·8
2·8
2·2
2·2
Hen 4175
4·4
3·1
3·3
3·2
3·3
Hen 4170
3·0
1·4
1·6 1·2
1·4
Hen 4176
3·2
2·1
2·7
2·7
Hen 4192
2·6
3·0
2·0
2·5
Hen 4160
1·6
5·8
2·6
3·0
Hen 4228
2·1
3·1
2·5
2·8
Maximal incremental change in plasma LH conc. (ng/ml)
Pre-injection plasma LH conc.(ng/ml)

was expected.

Progesterone injections between 4 h after one ovulation and 12 h before the next ovulation

As shown in Fig. 13a - d and 14, injections of 0.5 mg progesterone/kg between 4 h after an ovulation and 12 h before the next predicted ovulation resulted in similar changes in plasma LH levels. During the 15 to 30 min following an injection, plasma LH levels fell slightly (Fig. 13a - d). This may have been partially due to stress resulting from handling the birds, since a similar fall was noted after a control injection of the solution in which the progesterone was dissolved (Fig. 13e). LH levels rose steeply during the 30 to 90 minutes following an injection from a mean of 2.04 $\pm$ 0.12 (S.E.M.) ng/ml to a mean maximum of 3.62 $\pm$ 0.20 ng/ml (n = 37). LH levels started to fall soon after peak values had been reached, but were still above pre-injection levels 3.5 h after injection (Fig. 13a - d).

Progesterone injections 12 to 8 h before ovulation

When 9 hens were injected with progesterone 12 to 8 h before a predicted ovulation, i.e. 4 to 0 h before a pre-ovulatory LH surge, LH levels first fell slightly for 30 min and then rose during the next 30 min to a mean maximum of 2.47 $\pm$ 0.18 ng/ml (Fig. 13). They remained at around this level for the next 2.5 h after which blood samples were no longer taken. The mean maximal incremental change in LH levels (0.79 $\pm$ 0.12 ng/ml) was significantly lower ($P < 0.01$) than the change (1.60 $\pm$ 0.28 ng/ml) resulting from injection of the same hens at 4 or more hours after ovulation.

Progesterone injections 8 to 4 h before ovulation

When 12 hens were injected with progesterone 8 to 4 h before a predicted ovulation, at a time when endogenously induced pre-ovulatory LH surges were being or had been initiated, plasma LH levels rose immediately and rapidly with a latent period of less than 15 min to a mean maximal value of 4.37 ± 0.33 ng/ml within 45 min (Fig. 13b). The mean maximal incremental change (2.34 ± 0.20 ng/ml) was significantly greater ($P < 0.02$) than the change (1.61 ± 0.18 ng/ml) observed after the same hens had been injected 4 or more hours after ovulation. The rapid rise in LH levels observed after the injection of progesterone when pre-ovulatory LH levels were increasing was followed by a steep fall; LH levels were back to pre-injection values 3 to 3.5 h after injection. In contrast, LH levels were still above pre-injection values at this time in the same hens injected with progesterone 4 or more hours after ovulation (Fig. 13b).

Progesterone injections 4 to 0 h before ovulation

When hens were injected with progesterone 4 to 2 h ($n = 8$) and 2 to 0 h ($n = 8$) before a predicted ovulation, i.e. when pre-ovulatory LH levels were falling, there were generally no resulting increases in LH secretion (Fig. 13c, d). However, in 3 hens there were transient rises suggesting that at this time in some cases there was still a residual positive feedback response to progesterone (Fig. 15a, b).

An overall view of the variation in the mean maximal incremental changes in plasma LH levels in response to progesterone injected at various stages of the ovulatory cycle in the 45 hens used in this study is shown in Fig. 14. The change observed when pre-ovulatory LH levels were rising was significantly greater than that

Figure 13

Comparison of changes in the concentration of plasma LH in laying hens after single intramuscular injections of 0.5 mg progesterone/kg during various phases of the pre-ovulatory LH surge with those observed after the steroid was given at any time during the rest of the ovulatory cycle. The hens were injected with progesterone on two occasions. On the first (right hand side of the panel), the steroid was given between 4 h after and 12 h before an oviposition and on the second (left hand side of the panel) 12 to 8 h before oviposition, i.e. immediately preceding the pre-ovulatory LH surge (a), or 8 to 4 h before oviposition, i.e. when pre-ovulatory LH levels were rising (b), or 4 to 0 h before oviposition, i.e. when pre-ovulatory LH levels were falling (c, d). Vehicle-injected controls (e). P, time of progesterone injection. Vertical lines represent $\pm$ S.E.M.

Figure 14

Mean maximal incremental changes in the concentration of plasma LH after the single intramuscular injection of hens with 0.5 mg progesterone/kg at various times of the ovulatory cycle in relation to a predicted ovulation. The ovulatory cycle is taken to be of 26 h duration. Vertical lines represent $\pm$ S.E.M. For purposes of comparison the variation in pre-ovulatory LH levels is indicated by the dotted line (taken from Fig. 9a). * , these mean maximal incremental changes in LH were significantly different from each other (for all comparisons, $P < 0.001$).

Fig. 13

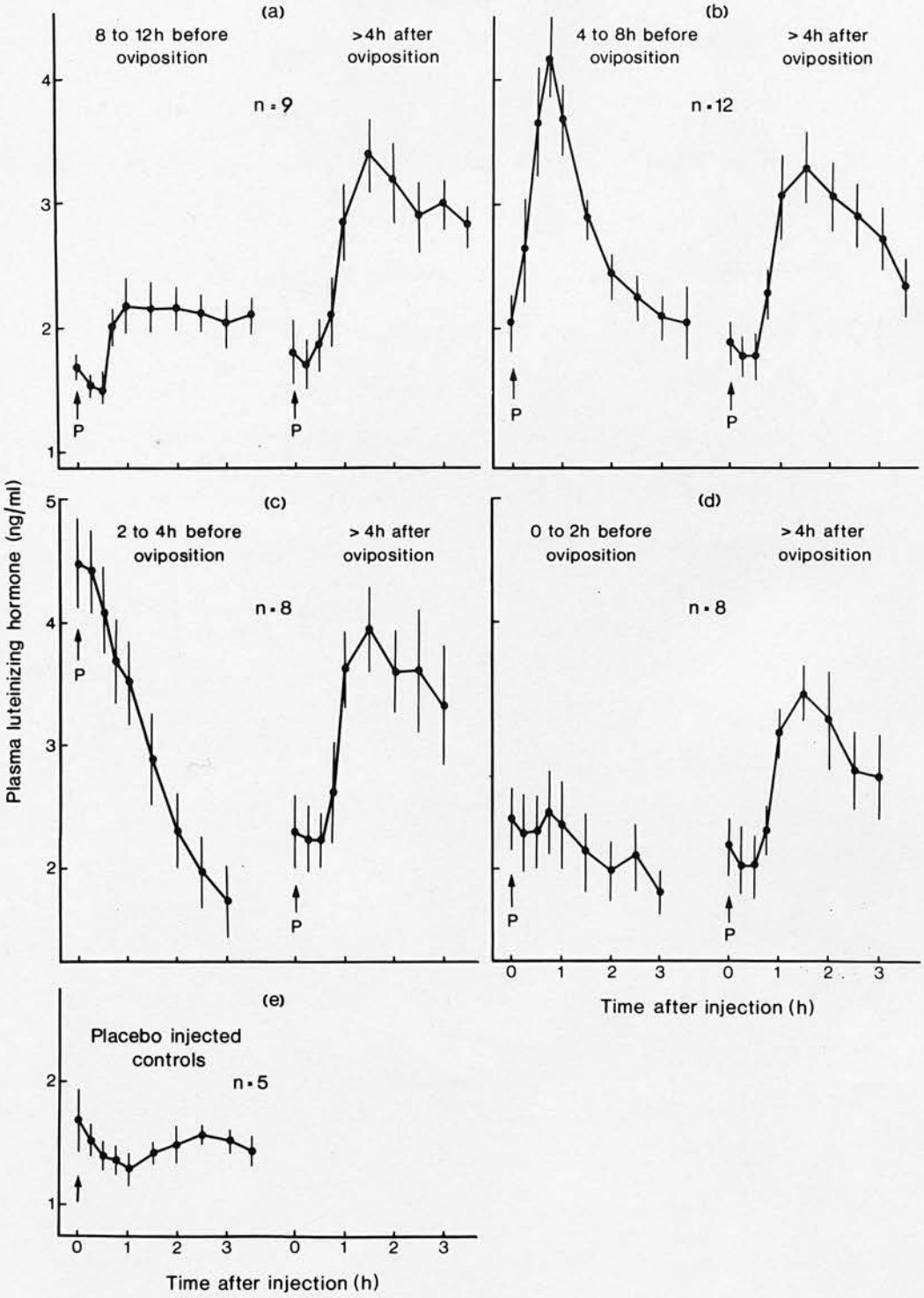
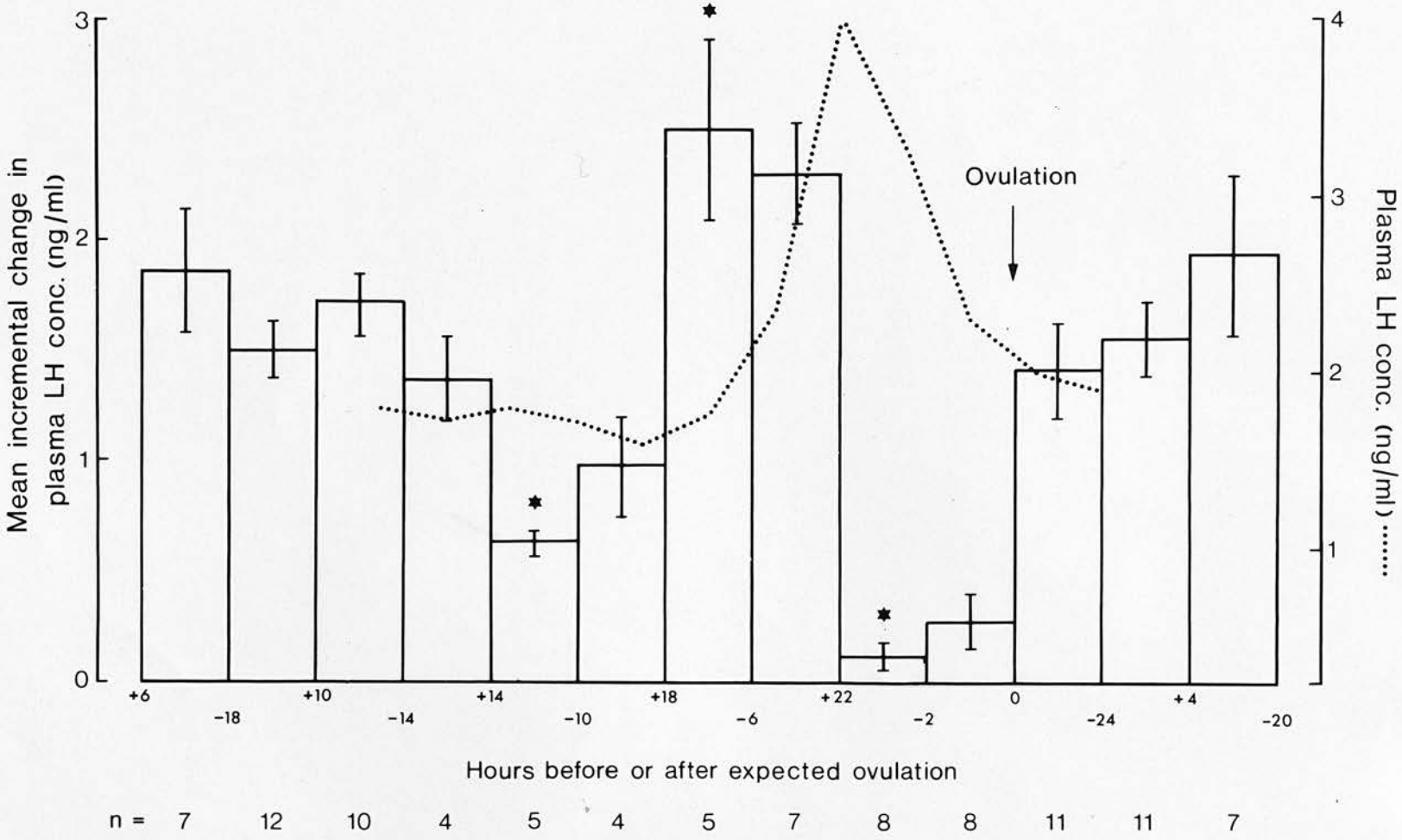

(a)
8 to 12h before oviposition
>4h after oviposition
n = 9
(b)
4 to 8h before oviposition
> 4h after oviposition
n = 12
(c)
2 to 4h before oviposition
> 4h after oviposition
n = 8
(d)
0 to 2h before oviposition
>4h after oviposition
n = 8
(e)
Placebo injected controls
n = 5
P
Plasma luteinizing hormone (ng/ml)
Time after injection (h)

Fig. 14


Mean incremental change in plasma LH conc. (ng/ml)
Plasma LH conc. (ng/ml) .....
Ovulation
0
1
2
3
4
+6
-18
+10
-14
+14
-10
+18
-6
+22
-2
0
-24
+4
-20
Hours before or after expected ovulation
n = 7 12 10 4 5 4 5 7 8 8 11 11 7

seen just before the levels started to rise ($P < 0.001$) or whilst they were falling ($P < 0.001$) (Fig. 14). Although the LH response to progesterone was depressed at the beginning of a spontaneous LH surge, it was significantly greater ($P < 0.001$) than after the steroid was injected when the surge had ended (Fig. 14).

Recovery of the positive feedback response to progesterone

The time required for the hypothalamo-hypophysial complex to regain its positive feedback response to progesterone, after an oviposition that was accompanied by an ovulation, varied in the 9 hens that were investigated (Figs. 12 and 15). When hens 4135 and 4154 were injected 51 min and 1 h 29 min, respectively, after an oviposition, there was only a transient rise in plasma LH (Fig. 15c, d). However, the pre-injection concentrations of LH were high, indicating that progesterone had been administered on the declining slope of a pre-ovulatory LH surge. In these cases, where the imminent ovulation was predicted to terminate a sequence, it is possible that ovulation occurred later than the usual 14 to 75 min (Warren & Scott, 1935) after oviposition. On the other hand, in hen 4228 injected with progesterone 12 min before oviposition, the pre-injection LH level was not high and there was a resulting maximal incremental change of 1.19 ng/ml in the circulating levels of LH (Fig. 15f). This response, however, was of a smaller magnitude and of a shorter duration than that observed in the same hen injected 5 h 10 min after oviposition (Fig. 15g). A similarly diminished LH response was also noted on all but three occasions when injections of progesterone were given within 2h 15 min of oviposition. When such hens were given the steroid 3 or 4 h after oviposition, the LH response was generally fully recovered

Figure 15

Changes in the concentration of plasma LH in individual hens given a single intramuscular injection of 0.5 mg progesterone/kg before (a, b, f) or after (c, d, e, g, h, i) an oviposition accompanied by an ovulation. In hens 4154 (d, e), 4228 (f, g) and 4170 (h, i) progesterone was injected close to the time of oviposition (d, f, h) and the resulting LH response is compared to that obtained after the steroid was given on another day, 3 to 5 h after oviposition (e, g, i). P, time of progesterone injection. Vertical lines represent 95 % confidence limits.

Fig. 15

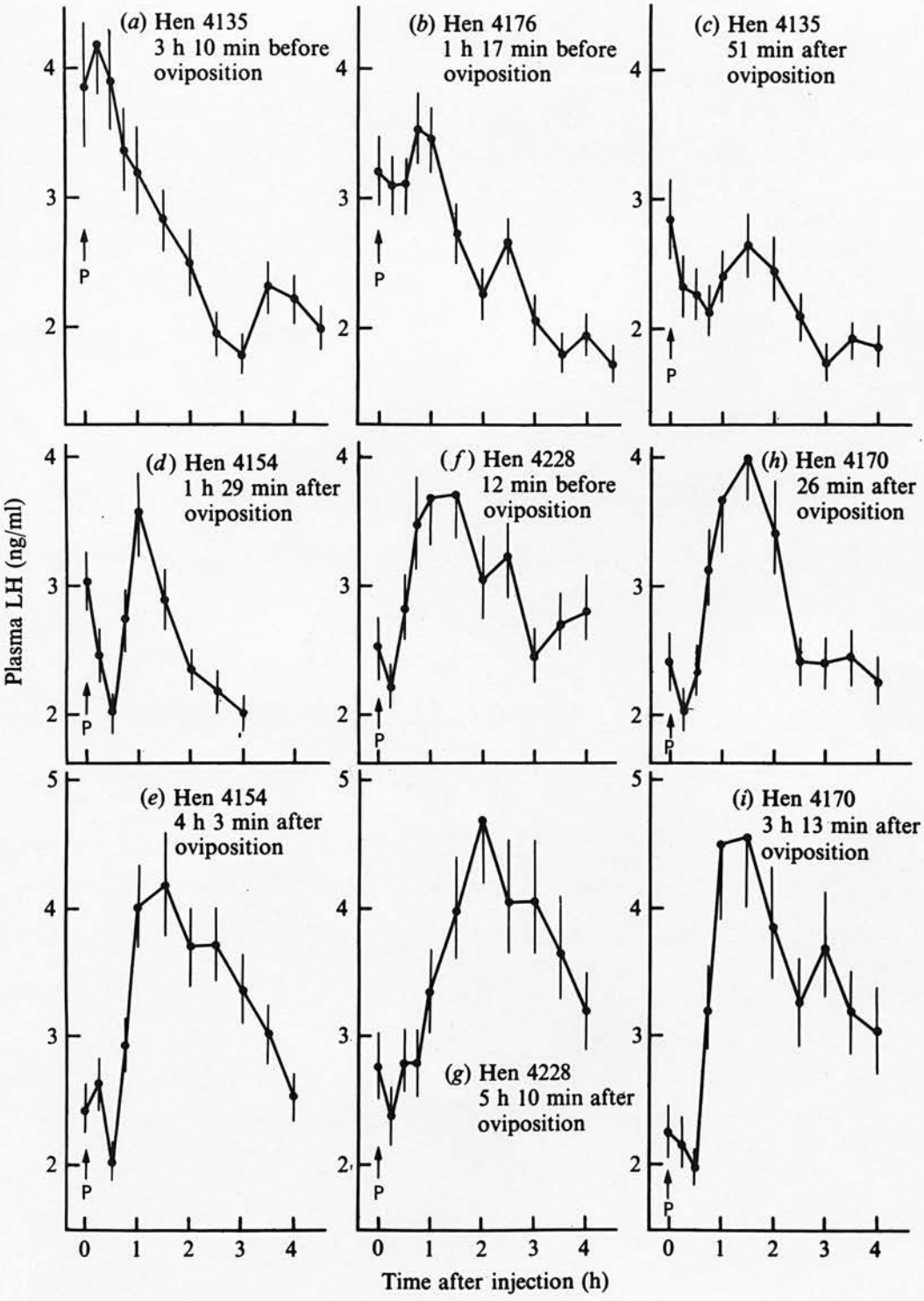
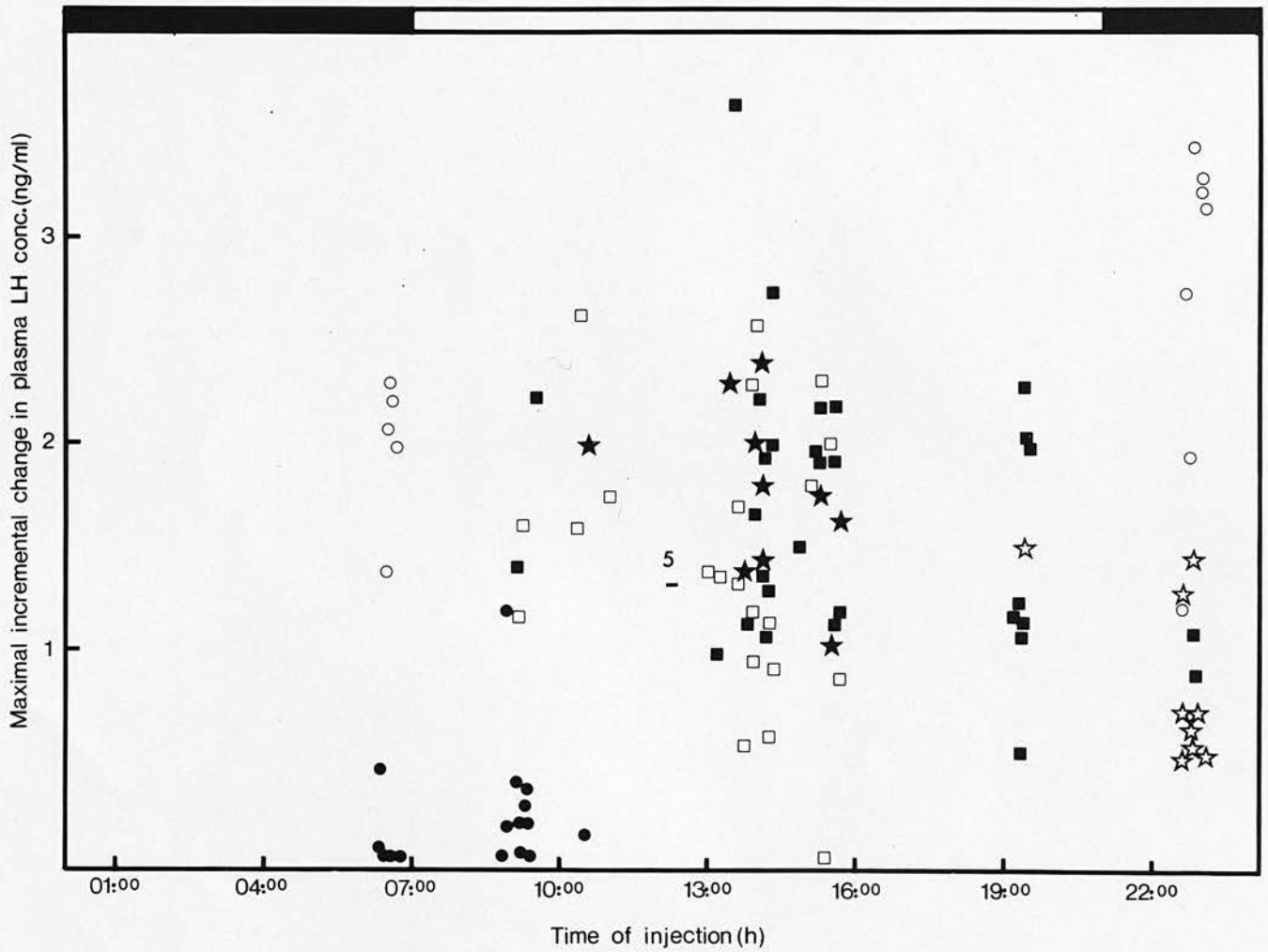

(a) Hen 4135
3 h 10 min before oviposition
(b) Hen 4176
1 h 17 min before oviposition
(c) Hen 4135
51 min after oviposition
(d) Hen 4154
1 h 29 min after oviposition
(f) Hen 4228
12 min before oviposition
(h) Hen 4170
26 min after oviposition
(e) Hen 4154
4 h 3 min after oviposition
(g) Hen 4228
5 h 10 min after oviposition
(i) Hen 4170
3 h 13 min after oviposition
P
Plasma LH (ng/ml)
Time after injection (h)

Figure 16

Comparison of maximal incremental changes in the concentration of plasma LH after a single intramuscular injection of 45 hens with 0.5 mg progesterone/kg at various stages of the ovulatory cycle and at various times of the day. ✡ , 12 to 8 h before; o , 8 to 4 h before; ● , 4 to 0 h before; □ , 0 to 4 h after; ■ , 4 to 14 h (-12 h) after an oviposition accompanied by an ovulation. ✱ , before or after an oviposition not accompanied by an ovulation. Black horizontal bars represent the hours of darkness.

Fig. 16


Maximal incremental change in plasma LH conc.(ng/ml)
3
2
1
5
01:00
04:00
07:00
10:00
13:00
16:00
19:00
22:00
Time of injection (h)

(e.g. hen 4154, Fig. 15d, e; hen 4170, Fig. 15h, i). In 4 hens a full LH response to progesterone was not observed until 4 h after oviposition.

The relationship between the maximal incremental changes in plasma LH concentration after an injection of 0.5 mg progesterone/kg and the time of day at which the steroid was given is shown in Fig. 16. Except when administered 4 to 0 h before an ovulation, progesterone injected at any time between 06:00 h and 23:00 h resulted in an increase in plasma LH levels. The magnitude of the LH response was related solely to the proximity of the progesterone injection to a predicted ovulation. There was no evidence to suggest that the magnitude of the response was influenced by the hour of day at which the steroid was injected.

(v) Effects of antiserum to progesterone on the progesterone-induced LH surge

While this study has shown that progesterone will cause a rapid release of LH when injected at any time during the ovulatory cycle apart from the 4 h preceding ovulation, the administration of antiserum to progesterone had been unable to completely block the natural pre-ovulatory LH surge (page 56). This may have resulted from the inability of the antiserum to neutralize the biological effects of the pre-ovulatory rise in progesterone secretion. Hence, the effectiveness of anti-progesterone to neutralize the positive feedback effects of injected progesterone on LH secretion was tested. Two laying hens were injected with 1.25 ml/kg of the same progesterone antiserum as was used in the earlier study (page 52) and one control hen with 1.25 ml/kg of the normal sheep serum. 4.25 h after injecting the antiserum or normal sheep serum, each hen was injected intramuscularly with 0.5 mg progesterone

/kg. All injections were given between 4 h after and 12 h before the time of a predicted ovulation. A blood sample was withdrawn immediately before injections of either antiserum or progesterone and at 15 or 30 min intervals after giving the progesterone (page 61).

In each hen, 0.5 mg progesterone/kg increased plasma LH levels, although the magnitude of the induced LH rise was less in the anti-progesterone treated hens, 0.87 ng/ml (89 % rise) and 1.26 ng/ml (52 % rise), than in the hen treated with normal sheep serum, 1.56 ng/ml (115 % rise).

It appeared that the anti-progesterone was only partially neutralizing the biological activity of the injected steroid, and it therefore seems likely that in the earlier study (page 56) there was still sufficient un-neutralized endogenous progesterone present to cause an LH surge.

b. Deoxycorticosterone (DOC)

One to two-year-old laying Shaver hens were injected intramuscularly with deoxycorticosterone acetate at various stages of the ovulatory cycle. The esterified steroid was dissolved in a solution of propylene glycol (page 31) and hens were injected at either 0 to 8 h after any ovulation (C_n), 8 to 9 h after the first ovulation (C_1), or 22 to 26 h after the final ovulation (C_t) of a sequence. LH responses to steroid injections at the beginning and end of the ovulatory cycle could thereby be compared. Plasma LH levels were measured in a blood sample of 0.75 ml taken by venepuncture at the time of injection, and in 11 more taken at 15 and 30 min intervals, as described on page 61.

In all but 5 of 25 hens injected with 0.1, 0.5 or 1.0 mg DOCA /kg between 0 and 9 h after an ovulation or 22 to 26 h after the

terminal ovulation of a sequence, there was a rise in plasma LH levels commencing within 1 h of injection. However, the magnitude of the incremental change in LH levels was very variable, ranging from 0.47 to 2.10 ng/ml and was not related to either the dose-level or the phase of the ovulatory cycle at which the steroid was injected.

c. Testosterone

The same solvent and frequency of blood sampling as were used in the above DOC study were also used for studies on the effects of testosterone, androstenedione, oestrone and oestradiol-17β on LH secretion.

The effects of testosterone injections on plasma LH levels varied according to the dose and the stage of the cycle at which the steroid was injected.

(i) Injections 0 to 8 h after any ovulation (C_n)

The mean change in plasma LH concentration after injection of 0.1, 0.5, 1.0 or 2.0 mg testosterone/kg between 0 and 8 h after ovulation is shown in Fig. 17. The mean change after injections of the carrier is shown in Fig. 18 d. In all cases, plasma LH levels tended to fall during the first 90 min after injection. However, the plasma LH concentration at 90 min was only significantly lower than the pre-injection value after injection of 0.1 ($P < 0.05$) and 0.5 ($P < 0.01$) mg testosterone/kg, and is not attributed to a negative feedback effect of testosterone on LH secretion. No consistent changes in plasma LH concentration occurred during the remaining 3.5 h of the sampling period.

(ii) Injections 8 to 9 h after C_1 ovulation

A similar depression in LH secretion to that described above

was observed after injections of 0.1, 0.5 or 1.0 mg testosterone/kg between 8 and 9 h after a C_1 ovulation (Fig. 17 ii). However, in no case was this depression found to be statistically significant. In contrast, after injections of 2.0 mg testosterone/kg plasma LH levels immediately began to rise and reached maxima after 1 h. The LH concentration at this time was significantly ($P < 0.05$) higher than the pre-injection value. The incremental change in LH level was 0.86 $\pm$ 0.25 (S.E.M.) ng/ml from the pre-injection value of 1.23 $\pm$ 0.30 ng/ml.

(iii) Injections 22 to 26 h after C_t ovulation

The LH response to injections of testosterone during the period between 9 and 22 h after ovulation was not investigated. Injection of 0.5, 1.0 and 2.0 mg testosterone/kg between 22 and 26 h after the final ovulation of a sequence resulted in significant ($P < 0.01$ in all cases) mean maximal incremental changes in LH levels of 1.98 $\pm$ 0.17 (S.E.M.) ng/ml, 2.17 $\pm$ 0.21 ng/ml and 2.41 $\pm$ 0.31 ng/ml respectively (Fig. 17b, c, d). The dose of 0.1 mg/kg failed to stimulate LH secretion (Fig. 17a) in all but one hen in which LH levels rose by a maximum of 0.77 ng/ml. The time taken for LH levels to start rising after injection was inversely related to the dose of testosterone; for doses of 0.5, 1.0 and 2.0 mg testosterone/kg, these intervals were respectively 2.5 $\pm$ 0.13 h, 1.4 $\pm$ 0.28 h and 0.7 $\pm$ 0.10 (S.E.M.) h. Plasma LH levels continued to rise for between 2 and 3 h in each case (Fig. 17 iii).

d. Androstenedione

Androstenedione was injected intramuscularly at dose levels of 0.1 (n = 3), 0.5 (n = 3) or 1.0 (n = 3) mg/kg at between 0 and 9 h after ovulation. Three hens were injected at each of the same dose-

Figure 17

Changes in the concentration of plasma LH in laying hens after single intramuscular injections of (a) 0.1, (b) 0.5, (c) 1.0, (d) 2.0 mg testosterone/kg at:-

(i) 0 to 8 h after any ovulation of a sequence (C_n),

(ii) 8 to 9 h after the first ovulation of a sequence (C_1),

(iii) 22 to 26 h after the terminal ovulation of a sequence (C_t).

Vertical lines represent ± S.E.M.

Figure 18

Changes in the concentration of plasma LH in laying hens after single intramuscular injections of :-

(a) 0.01, 0.1 or 1.0 mg oestrone/kg,

(b) 0.01, 0.1 or 1.0 mg oestradiol-17β/kg,

(c) 0.1, 0.5 or 1.0 mg androstenedione/kg,

at either ■——■ 0 to 9 h after any ovulation of a sequence, or □---□ 22 to 26 h after the terminal ovulation of a sequence.

(d) Controls injected with the carrier at 0 to 9 h after ovulation.

Vertical lines represent ± S.E.M.

Fig. 17

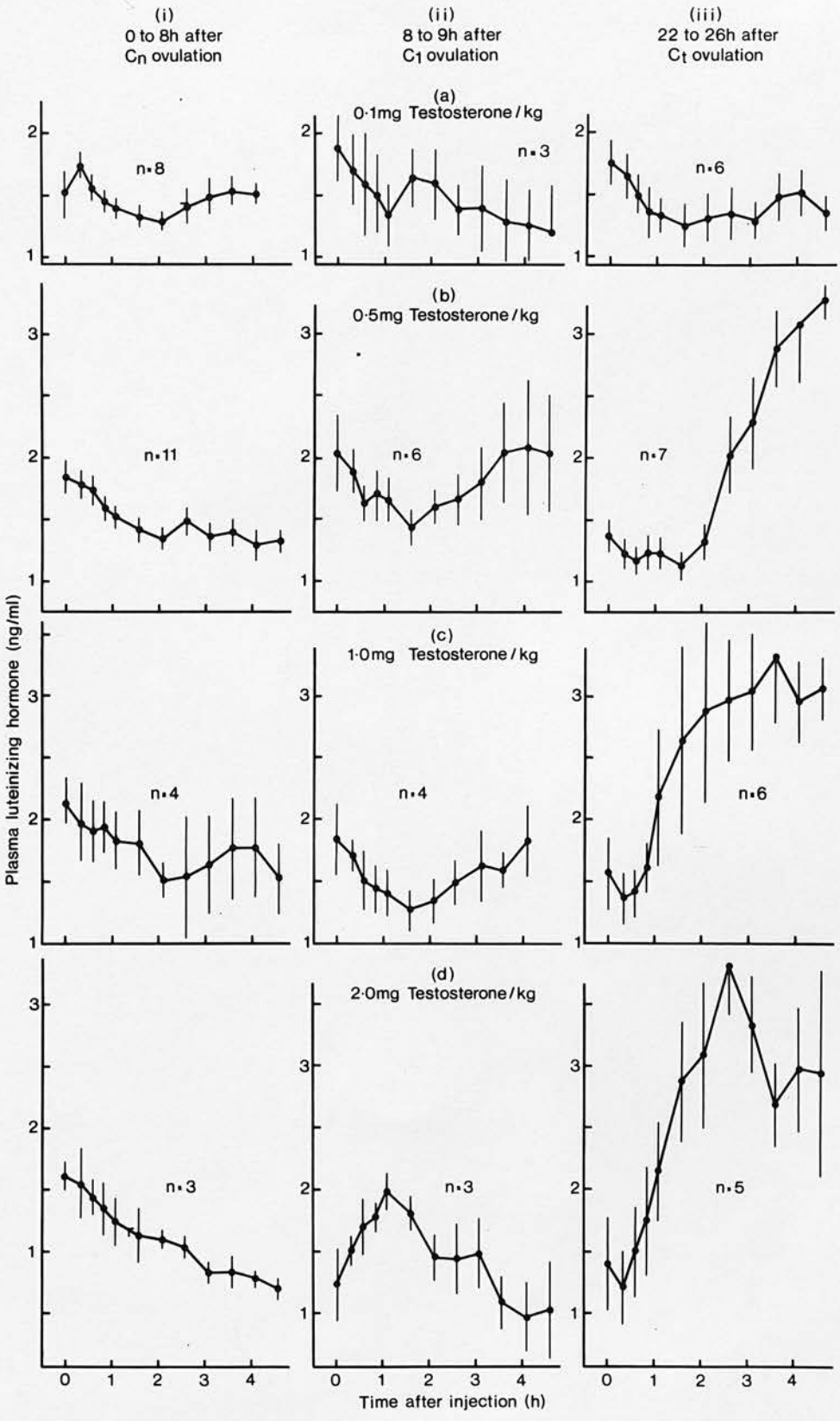
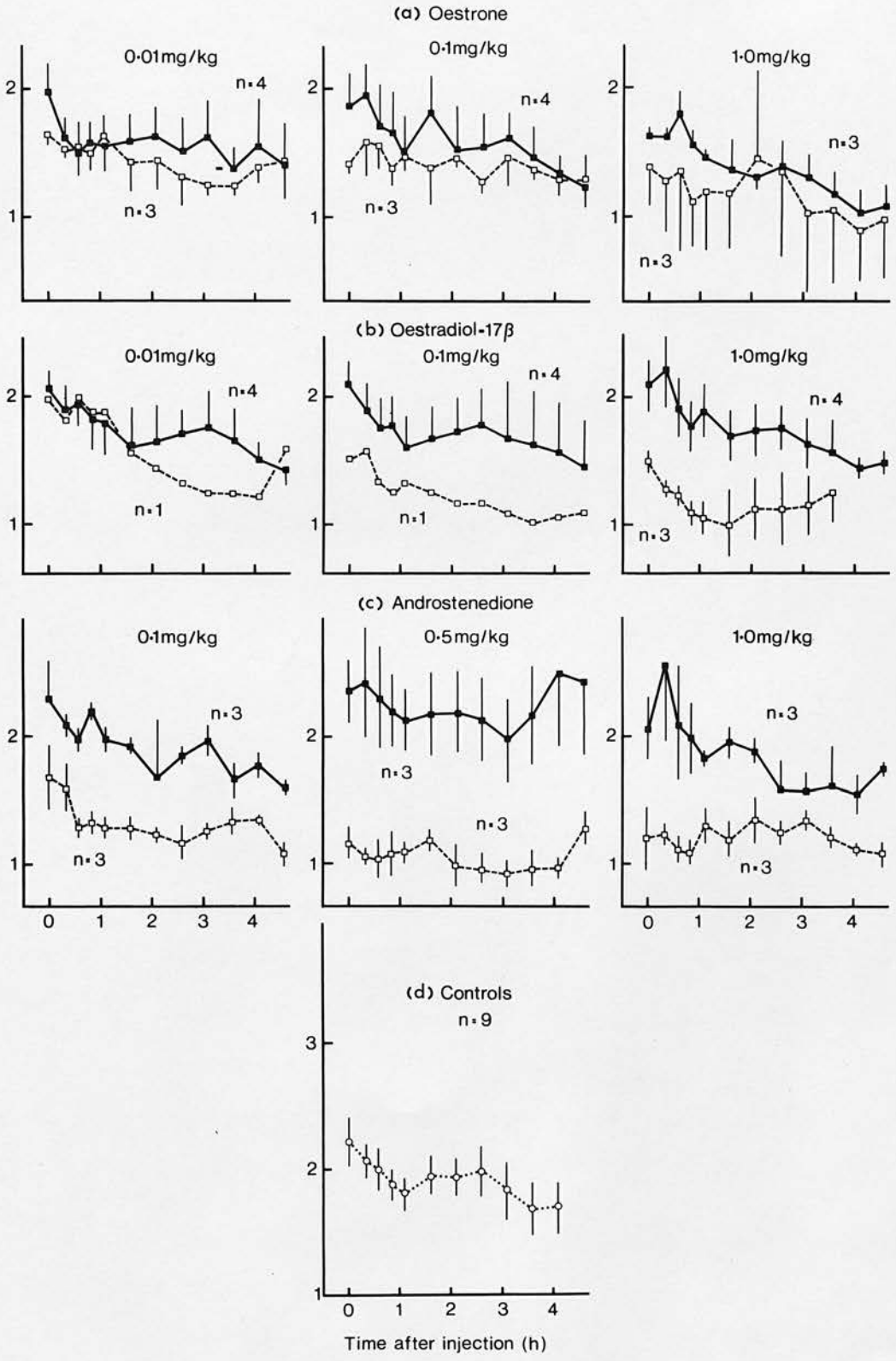

(i)
0 to 8h after
Cn ovulation
(ii)
8 to 9h after
C1 ovulation
(iii)
22 to 26h after
Ct ovulation
(a)
0·1mg Testosterone / kg
(b)
0·5mg Testosterone / kg
(c)
1·0mg Testosterone / kg
(d)
2·0mg Testosterone / kg
n = 8
n = 3
n = 6
n = 11
n = 6
n = 7
n = 4
n = 4
n = 6
n = 3
n = 3
n = 5
Plasma luteinizing hormone (ng/ml)
Time after injection (h)

Fig. 18


(a) Oestrone
0·01mg/kg
n·4
n·3
0·1mg/kg
n·4
n·3
1·0mg/kg
n·3
n·3
(b) Oestradiol-17β
0·01mg/kg
n·4
n·1
0·1mg/kg
n·4
n·1
1·0mg/kg
n·4
n·3
(c) Androstenedione
0·1mg/kg
n·3
n·3
0·5mg/kg
n·3
n·3
1·0mg/kg
n·3
n·3
(d) Controls
n·9
Time after injection (h)

levels between 22 and 26 h after the terminal ovulation of a sequence. In each case, plasma LH levels tended to fall during the first 90 min after injection but never significantly lower than the pre-injection value (Fig. 18c). A similar response was observed in the 9 control injected hens (Fig. 18d); the extent to which plasma LH levels were depressed was not related to the dose of androstenedione.

e. Oestrone and oestradiol-17β

Oestrone or oestradiol-17β were injected into groups of 3 or 4 hens at dose-levels of 0.01, 0.1 and 1.0 mg/kg between 0 and 9 h after ovulation. Either 1 or 3 hens were injected at the same dose-levels between 22 and 26 h after the terminal ovulation of a sequence. Neither oestrogen stimulated LH secretion during these two periods of the ovulatory cycle and the pattern of LH secretion after injection was similar to that of the control hens (cf. Figs. 18a, b, and d).

Conclusion

It was concluded that of the steroids which are known to be secreted in increased quantities at about the same time as the pre-ovulatory LH peak, progesterone induces LH release when injected at any stage of the ovulatory cycle, apart from the 4 h preceding ovulation. Injections of oestrone and oestradiol-17β failed to stimulate LH secretion, while testosterone stimulated LH secretion at 22 to 26 h, but not at 0 to 8 h after ovulation.

why the difference between testo and progesterone.

f. Modification of progesterone-induced LH release by gonadal steroids

The previous study has shown that of the steroids which are known to be present in increased concentrations in the circulation between 9 and 4 h before ovulation (see page 23), only progesterone

and testosterone can exert a positive feedback effect on LH release. While the pre-ovulatory rise in oestrogen has been found to precede that of LH by about 1 h (Senior & Cunningham, 1974), the previous study suggested (page 71) that rising levels of oestrogen do not cause the pre-ovulatory LH surge. However, it is possible that oestrogen may partly inhibit the LH-releasing effects of progesterone. This is suggested by the observation that a comparatively small LH response was observed after the injection of progesterone between 4 and 0 h before the start of the natural pre-ovulatory LH surge (page 64), at a time when endogenous oestrogen levels in the circulation may already be rising (Senior & Cunningham, 1974). This possibility was investigated experimentally.

(i) Effects of oestrogen injections on progesterone-induced LH surges.

Six hens were injected intramuscularly 2 h or more after an oviposition, with either 0.01, 0.1 or 1.0 mg oestradiol benzoate/kg, followed 1 h later by a single injection of 0.1 mg progesterone/kg. This interval of 1 h was estimated to allow sufficient time for oestrogen levels to rise in the circulation. Two further groups of 4 or 5 hens were treated similarly with a combination of respectively 0.1 mg oestradiol benzoate/kg followed by 0.5 mg progesterone/kg, or 1.0 mg oestradiol benzoate/kg followed by 0.5 mg progesterone/kg. A blood sample was withdrawn by venepuncture from a wing vein immediately before each of the injections and samples were taken 3 to 4 h after the second injection at 15 and 30 min intervals as described on page 61. The changes in plasma LH concentration following these two treatments are shown in Fig. 19a and b. Fig. 19b (i) shows the mean LH response

in 37 hens given a single injection of 0.5 mg progesterone/kg in an earlier experiment (page 63); they were used as controls in this experiment.

No consistent changes were observed in LH secretion 1 h after injection of 0.01, 0.1 or 1.0 mg oestradiol benzoate/kg (Fig. 19a, b), and there was no evidence to suggest that the rise in circulating oestrogen resulting from these injections could suppress the progesterone-induced LH surge. There was considerable variation in the magnitude of the LH response to injections of 0.1 mg progesterone/kg (range 0.6 to 4.1 ng/ml (Fig. 19a i - iii)) after oestrogen injection. However, a wide range in magnitude of response (0.6 to 2.6 ng/ml) was also observed in a previous experiment in 7 hens injected with 0.1 mg progesterone/kg alone (see page 61).

The mean LH responses resulting from injections of 0.1 or 1.0 mg oestradiol benzoate/kg followed by 0.5 mg progesterone/kg (Fig. 19b ii, iii) were similar to the mean response of 37 hens given a single injection of 0.5 mg progesterone/kg (Fig. 19b i).

(ii) Effects of progesterone injections on progesterone-induced LH surges

In an earlier study it was found that the LH response to 0.5 mg progesterone/kg injected when pre-ovulatory LH levels were rising consisted of an immediate, accelerated increase in LH secretion followed by a steep fall (page 65). Studies in mammals (see page 178) have shown that the secretion of LH in response to injected LH-RH is enhanced when pre-ovulatory LH levels are rising. This is thought to be due to an increase in pituitary responsiveness to LH-RH as a result of rising levels of gonadal steroids in the circulation. The possibility

that the enhanced LH response to progesterone at this stage of the hen ovulatory cycle is due to rising blood levels of progesterone was tested experimentally.

Pre-treatment with progesterone 50 min earlier

Eight hens were injected intramuscularly with either 0.05, 0.1 or 0.5 mg progesterone/kg, followed 50 min later by a second injection of 0.5 mg progesterone/kg. Plasma LH levels were measured in blood samples taken by venepuncture at 15 and 30 min intervals after the second progesterone injection for 3 to 4 h (Fig. 19c i - iii).

Fifty minutes after the initial injection of 0.05, 0.1 or 0.5 mg progesterone/kg, plasma LH levels had risen by means of 0.17 ± 0.20 (S.E.M.) ng/ml (n=3), 0.12 ± 0.16 ng/ml (n=3) and 0.52 ± 0.11 ng/ml (n=2) respectively. It can therefore be assumed that the second progesterone injection was given while the first was exerting a positive feedback effect on LH secretion. In birds pre-treated with 0.05, 0.1 and 0.5 mg progesterone/kg, the second injection of 0.5 mg progesterone/kg caused mean rises within 15 min of 1.07 ± 0.37 (S.E.M.) ng/ml (n=3), 1.96 ± 0.31 ng/ml (n=3) and 0.59 ± 0.05 ng/ml (n=2) respectively (Fig. 19c). This contrasts with the LH response following a single injection of 0.5 mg progesterone/kg in untreated hens (Fig. 19b i), where LH levels had fallen by a mean of 0.07 ng/ml 15 min after injection. It is apparent that pre-treatment with progesterone 50 min earlier enabled the positive feedback system to respond immediately to a second progesterone injection. The maximal incremental changes in plasma LH levels resulting from the second injection of progesterone in hens pre-treated with 0.05, 0.1 and 0.5 mg progesterone/kg were, respectively, 2.49 ± 0.29 (S.E.M.) ng/ml,

2.41 ± 0.15 ng/ml and 1.20 ± 0.42 ng/ml, and were reached at 63 ± 13 min, 63 ± 16 min and 37 ± 7 min after injection.

It was noted that in hens pre-treated with 0.5 mg progesterone /kg plasma LH levels first rose to a peak but subsequently fell very rapidly to pre-injection values within 1.5 and 2.5 h after the second progesterone injection (Fig. 19c iii). This rate of fall was much greater than was normally seen after giving a single injection of progesterone (Fig. 19b i) and suggests that where the concentration of this steroid in the blood is high, it may inhibit the positive feedback effects of further increases in the levels of circulating progesterone. If this is so, the inability of progesterone to cause an LH surge on the declining slope of the pre-ovulatory LH peak (see page 64) could have been due to the high blood level of progesterone associated with the LH peak.

Pre-treatment with progesterone 2.5 h earlier

To investigate this possibility, a group of 8 hens were injected intramuscularly with 0.5 mg progesterone/kg between 0.5 h and 21.0 h after the occurrence of an oviposition/ovulation, and another group of 5 hens were injected between 2.0 and 0.2 h before an oviposition, presumably on the declining slope of a pre-ovulatory LH peak. In each case a second injection of 0.5 mg progesterone/kg was given 2.5 h later. A blood sample was taken by venepuncture immediately before each injection, and then further samples were taken at 15 and 30 min intervals (see page 61) for a further 3.5 h. The mean changes in plasma LH concentration following this treatment are shown in Fig. 19d i, ii.

In the 8 hens injected with progesterone after oviposition/

ovulation plasma LH levels 2.5 h later were 0.6 ± 0.15 (S.E.M.) ng/ml above the pre-injection values of 1.7 ± 0.14 ng/ml (Table 3b). Assuming that the pattern of LH secretion in this group of hens was similar to that shown in Fig. 19b i, for hens similarly treated with 0.5 mg progesterone/kg, then the second progesterone injection should have been given on the declining slope of the progesterone-induced LH surge. After the second injection, there was a transient rise of 0.19 ± 0.08 ng/ml, and thereafter the LH concentration gradually declined to the level observed at the beginning of the experiment (Fig. 19d i). In contrast, 2.5 h after injecting the 5 hens just before oviposition/ovulation, and therefore on the declining slope of the pre-ovulatory LH peak, plasma LH levels had fallen by 1.12 ± 0.31 (S.E.M.) ng/ml (n = 5) from 3.4 ± 0.30 ng/ml to 2.3 ± 0.33 ng/ml (Fig. 19d ii). It therefore appears that, as shown previously (page 65) progesterone injected on the declining LH slope did not induce an LH surge. However, in each of the 5 hens following the second injection of progesterone, there was a rise in plasma LH levels of between 0.24 and 1.10 ng/ml (mean, 0.75 ± 0.15 (S.E.M.) ng/ml) (Fig. 19d ii).

As demonstrated in Table 3, the maximal incremental changes in plasma LH levels following the second progesterone injection were related to the time before or after an oviposition/ovulation when the first injection was given, and also to the changes in plasma LH levels occurring between the first and second progesterone injections.

Since in both groups of hens (Fig. 19d i, ii) equal concentrations of progesterone had been injected, the inability of a second injection of progesterone to induce an LH surge appeared not to be wholly due to a high blood concentration of progesterone, but

Figure 19

Individual or mean changes in the concentration of plasma LH :-

(a) after an intramuscular injection of 0.1 mg progesterone/kg, preceded 1 h earlier by an intramuscular injection of (i) 0.01, (ii) 0.1 or (iii) 1.0 mg oestradiol benzoate/kg;

(b) after an intramuscular injection of 0.5 mg progesterone/kg (i) alone, or preceded 1 h earlier by an intramuscular injection of (ii) 0.1, or (iii) 1.0 mg oestradiol benzoate/kg;

(c) after an intramuscular injection of 0.5 mg progesterone/kg, preceded 50 min earlier by an intramuscular injection of (i) 0.05, (ii) 0.1 or (iii) 0.5 mg progesterone/kg.

(d) after an intramuscular injection of 0.5 mg progesterone/kg, preceded 2.5 h earlier by an intramuscular injection of 0.5 mg progesterone/kg at

(i) 0.5 to 21.0 h after an oviposition accompanied by an ovulation,

(ii) 2.0 to 0.2 h before an oviposition accompanied by an ovulation.

Vertical lines represent $\pm$ S.E.M.

Fig. 19

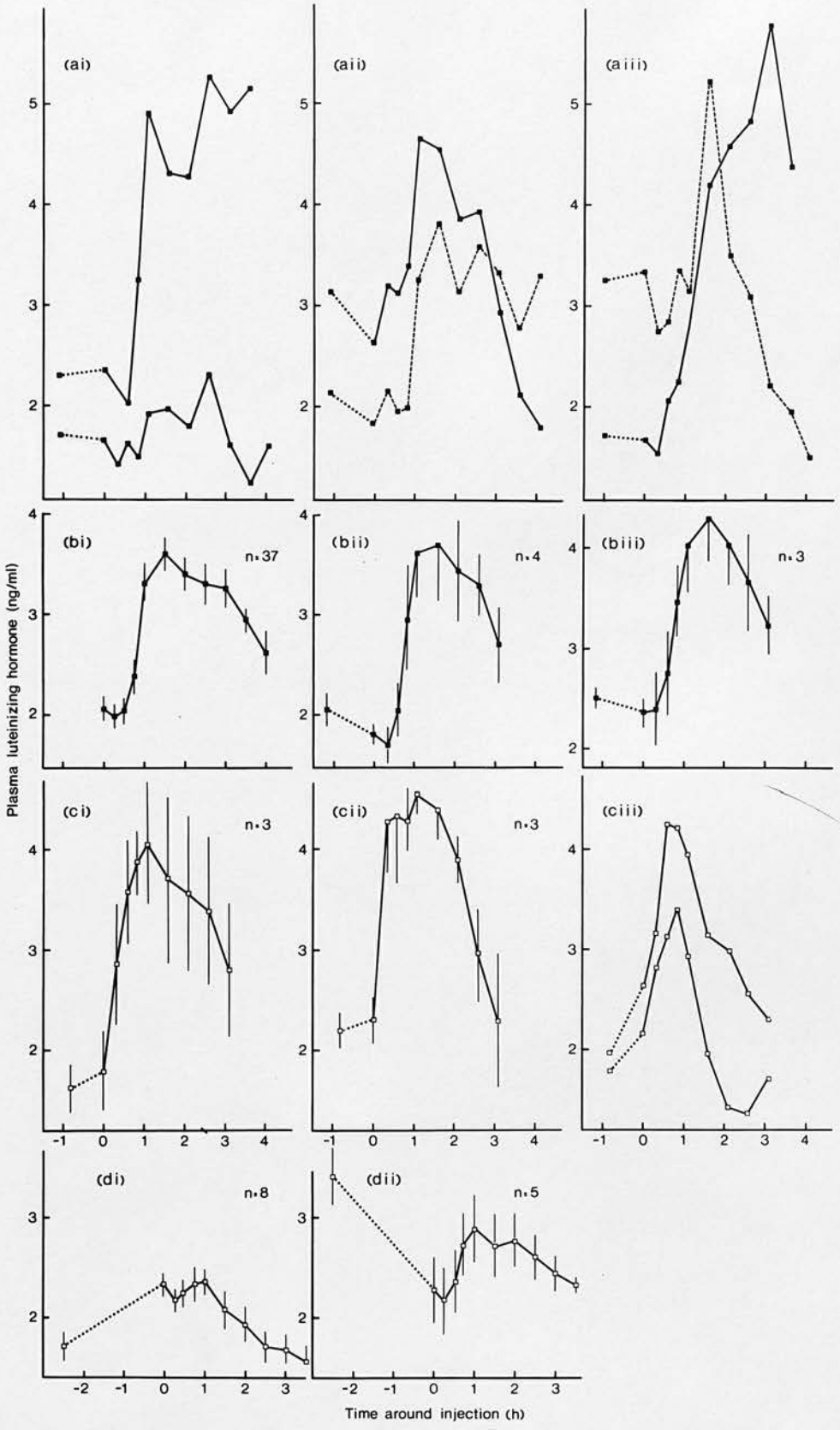

(ai)
(aii)
(aiii)
(bi)
n.37
(bii)
n.4
(biii)
n.3
(ci)
n.3
(cii)
n.3
(ciii)
(di)
n.8
(dii)
n.5
Plasma luteinizing hormone (ng/ml)
Time around injection (h)

Table 3. Changes in plasma LH levels following a second injection of progesterone in hens injected 2.5 h previously with 0.5 mg progesterone/kg either (a) shortly before or (b) after an oviposition/ovulation.

Time of 1st progesterone injection in relation to oviposition	Plasma LH levels at time of 1st progesterone injection (ng/ml)	Plasma LH levels at time of 2nd progesterone injection (ng/ml)	Rise (+) or fall (-) in plasma LH levels (ng/ml)	Max. incr. change in plasma LH after 2nd progesterone injection (ng/ml)
	(a) Injections before oviposition			
- 2.0 h	3.76	2.19	- 1.57	0.97
- 2.0 h	3.52	1.51	- 2.01	0.67
- 2.0 h	3.07	1.97	- 1.10	1.10
- 0.8 h	2.43	2.21	- 0.22	0.76
- 0.2 h	4.20	3.51	- 0.69	0.24
	(b) Injections after oviposition			
+ 0.5 h	2.08	2.12	+ 0.04	0.10
+ 0.2 h	1.29	2.32	+ 1.03	0.14
+ 0.3 h	1.63	2.69	+ 1.06	0.53
+ 0.5 h	1.63	1.87	+ 0.24	0.59
+ 2.3 h	1.25	2.00	+ 0.75	0.05
+ 3.0 h	1.66	2.73	+ 1.07	No response
+ 6.6 h	2.48	2.58	+ 0.10	0.11
+ 21.0 h	1.71	2.21	+ 0.50	No response

depended to some extent on whether or not there had been a recent progesterone-induced LH surge.

Conclusion

In conclusion, the injection of various dose-levels of oestradiol benzoate did not suppress the discharge of LH induced by an injection of progesterone 1 h later. The progesterone-induced LH discharge appeared to be facilitated by pre-treatment with progesterone 0.75 h earlier, while the effects of pre-treatment with progesterone 2.5 h earlier appeared to depend on whether or not that injection had been given during or after the time when pre-ovulatory LH levels were falling.

3. Development of the positive feedback response to progesterone during sexual maturation.

The development of the positive feedback response to progesterone during sexual maturation was investigated in relation to changes in the responsiveness of the pituitary to synthetic LH-RH.

Summary of experiments

d. Sensitivity to the negative feedback effects of oestrogen in immature hens. 85

e. Effects of priming injections of progesterone on the development of the positive feedback response. 87

Changes in plasma LH concentrations were measured after single injections of synthetic LH-RH and progesterone at various stages of sexual development. Synthetic LH-RH dissolved in 0.3 to 0.5 ml of 1 % saline was injected through a cannula into a wing vein at a dose level of 10 µg/kg body weight. A blood sample of 0.75 ml was taken through the same cannula immediately before injecting the releasing hormone and thereafter blood was withdrawn at 2-min intervals for 12 min, and then at 17, 22, 32 and 42 min after the injection. Forty-eight hours later, crystalline progesterone, dissolved in a propylene glycol solution (page 31) was injected intramuscularly after taking a pre-injection blood sample of 0.75 ml by venepuncture from the wing vein. Thereafter, another 12 blood samples were taken at 15- and 30-min intervals (page 61) for 5 h.

The investigation consisted of two series of experiments. In the first, a "cross-sectional" approach was used in which the LH responses to LH-RH and progesterone were studied in 7 groups of 4 to 13 pullets categorized according to the weights of their ovaries and oviducts. A total of 58 birds were injected with progesterone of which 40 had been injected 48 h previously with LH-RH.

The second series of experiments was a "longitudinal" study in which the same 10 pullets were injected with LH-RH and progesterone every 14 days from the age of 14 weeks until the first eggs were laid. Again, the injections of LH-RH and progesterone were separated by an

interval of 48 h. Blood samples of 1 ml were taken by venepuncture from a wing vein of each bird every 3 to 7 days during the experiment to measure the changes in basal level of LH in the circulation. A record was kept of the number of eggs laid by each hen during the first 6 months of lay. Variations in comb sizes (height x length, mm) and body weight (kg) were noted in both series of experiments.

Stages of sexual development

The process of sexual maturation was divided into seven stages designated I to VII (Table 4; Fig. 20). The ovaries and oviducts in birds at Stage 1 showed no signs of development whilst those in hens at Stage VII were fully functional. Ovarian and oviducal growth started at Stage II in conjunction with increased LH secretion. At Stage III, mean levels of LH in the circulation had reached a maxima and small 1 to 4 mm diameter white follicles had differentiated in the ovary. Between Stages III and V the oviducts grew more rapidly than the ovaries and there was a progressive fall in mean plasma LH concentrations. An increased rate of yolk deposition and the appearance of yellow yolky follicles of up to 15 mm diameter resulted in a rapid increase in ovarian weight at Stage V. Stage VI was characterized by rapidly growing yellow yolky follicles with diameters of up to 26 mm.

a. "Cross-sectional" study of the effects of 10 µg synthetic LH-RH/kg and 0.5 mg progesterone/kg on plasma LH levels during sexual development.

Control injections, at Stages I to V of sexual development, of the solution in which the LH-RH or progesterone (Fig. 21) was dissolved resulted in an immediate fall in plasma LH levels. This

Figure 20

Changes in the concentration of plasma LH during Stages I to VII of sexual maturation in relation to increases in ovarian and oviducal weights. Vertical lines represent $\pm$ S.E.M.

Fig. 20

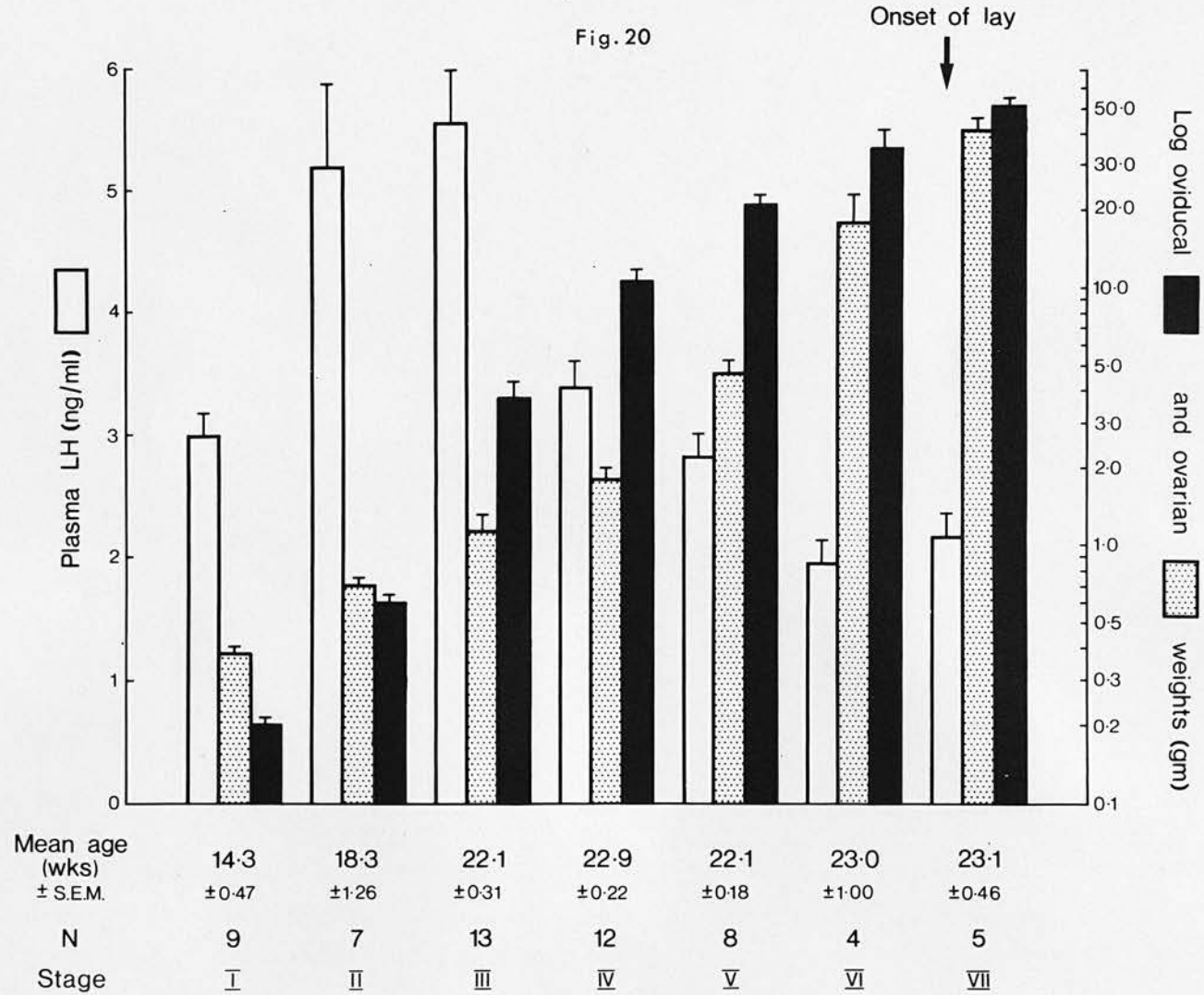

Onset of lay
Plasma LH (ng/ml)
Log oviducal and ovarian weights (gm)
0
1
2
3
4
5
6
0·1
0·2
0·3
0·5
1·0
2·0
3·0
5·0
10·0
20·0
30·0
50·0
Mean age (wks)
± S.E.M.
14·3 ±0·47
18·3 ±1·26
22·1 ±0·31
22·9 ±0·22
22·1 ±0·18
23·0 ±1·00
23·1 ±0·46
N
9
7
13
12
8
4
5
Stage
I
II
III
IV
V
VI
VII

Table 4. Classification of stages of sexual maturation in the domestic hen (means ± S.E.M.)

Stage of sexual maturation	No. of hens	Age (wks)	Body wt (kg)	Comb size: height x length (mm)	Ovarian (OV) & Oviducal (OD) wt (g)	Diameter of largest follicles (mm) and range	Pre-priming plasma LH (ng/ml)
I	9	14.3 ± 0.5	1.2 ± 0.1	286 ± 39	0.4 ± 0.0 (OV) 0.2 ± 0.0 (OD)	--	3.0 ± 0.2
II	7	18.3 ± 1.3	1.5 ± 0.1	524 ± 84	0.7 ± 0.1 (OV) 0.6 ± 0.1 (OD)	--	4.9 ± 0.6
III	13	22.1 ± 0.3	1.9 ± 0.1	968 ± 74	1.1 ± 0.2 (OV) 3.7 ± 0.6 (OD)	2 ± 0.8 (1-4)	5.5 ± 0.3
IV	12	22.9 ± 0.2	1.9 ± 0.1	1304 ± 117	1.8 ± 0.1 (OV) 10.8 ± 1.1 (OD)	5 ± 0.6 (4-8)	3.4 ± 0.2
V	8	22.1 ± 0.2	2.0 ± 0.1	1377 ± 244	4.7 ± 0.5 (OV) 20.9 ± 1.7 (OD)	13 ± 1.0 (7-15)	2.8 ± 0.2
VI	4	23.0 ± 1.0	1.8 ± 0.1	1138 ± 481	17.9 ± 4.4 (OV) 35.7 ± 5.5 (OD)	23 ± 2.0 (18-26)	2.0 ± 0.2
VII	5	23.1 ± 0.5	2.1 ± 0.1	1816 ± 382	40.7 ± 4.0 (OV) 51.3 ± 1.3 (OD)	28 ± 0.7 (26-30)	2.2 ± 0.2

inhibition of LH secretion was thought to be due to the stress resulting from handling (page 47). A positive response to progesterone was considered to have occurred if there was a sustained rise in plasma LH levels for at least 1 h. Any stimulation of LH secretion resulting from an injection of progesterone was expressed as the maximal incremental change in plasma LH levels measured from the point at which they first began to rise.

Stage I of development

The mean plasma LH level in hens at Stage I of development was 3.0 $\pm$ 0.2 (S.E.M.) ng/ml (n = 9). Following an injection of progesterone, LH levels fell for about 1 h and remained depressed except for abrupt random fluctuations during the next 4 h (Fig. 21). After this time blood samples were no longer taken. The occurrence of these fluctuations could not be related consistently to the time of progesterone injection and were thought to be the consequence of the episodic manner in which LH is secreted. Plasma LH levels rose within 2 min of injecting LH-RH, and at 7 min reached a mean maximal incremental change of 23.5 $\pm$ 5.6 ng/ml (n = 9). Thereafter LH levels declined, but were still elevated at 40 min after injection (Fig. 22; Table 5).

Stage II of development

At Stage II of sexual maturation the mean plasma LH level, 4.9 $\pm$ 0.6 ng/ml (n = 7), was significantly higher ($P < 0.001$) than in hens at Stage I of sexual development (Table 4). Immediately after an injection of progesterone LH levels fell, but about 2.5 h later consistently rose to form a small peak (Fig. 21). The highest peak LH values occurred at a mean time of 4.2 $\pm$ 0.4 h after progesterone

injection: the mean maximal incremental change in LH level was 1.2 ± 0.1 ng/ml (Table 5). When mean changes in plasma LH levels after an injection of progesterone were calculated for the group as a whole, the duration and magnitude of the response was not representative of that shown by individuals. This reflects individual variation in the time taken for progesterone to stimulate LH secretion, and the differences in mean basal plasma LH levels (Fig. 21). The mean concentration of LH in the blood rose by a mean maximum of 26.2 ± 7.7 ng/ml (n=5) at 6 min after injection of LH-RH (Fig. 22, Table 5).

Stage III of development

The plasma LH levels (mean, 5.5 ± 0.3 ng/ml (n=13)) in the hens killed at Stage III of sexual development had probably reached pre-pubertal maxima (Table 4, Fig. 20). When these birds were injected with progesterone, LH levels first fell and after a mean interval of 1.7 ± 0.2 h began to rise steeply to form a peak (Fig. 21). The mean maximal incremental change in plasma LH levels, 2.3 ± 0.2 ng/ml, was significantly greater ($P < 0.001$) than the response to progesterone observed in hens at Stage II of sexual development (Table 5). Peak levels of LH occurred at a mean interval of 2.9 ± 0.2 h after progesterone injection (Table 5). The mean maximal incremental change in plasma LH levels (13.0 ± 1.1 ng/ml) after injecting 9 hens with LH-RH was significantly lower ($P < 0.001$) than the response observed at Stage II of sexual development (Fig. 22, Table 5).

Stage IV of development

Mean plasma LH levels (3.4 ± 0.2 ng/ml (n=12)) in hens at Stage IV of development were significantly lower ($P < 0.001$) than in those at Stage III (Table 4). After injections of progesterone and

a mean latent period of 1.5 ± 0.1 h, plasma LH levels began to rise to a low plateau (Fig. 21). The mean maximal incremental change in LH levels, 1.2 ± 0.1 ng/ml, was significantly lower ($P < 0.001$) than that observed in birds at Stage III of sexual development (Table 5). Similarly there was also a significant reduction ($P < 0.001$) in the amount of LH released (mean, 5.1 ± 0.8 ng/ml (n=6)) after injections of LH-RH (Fig. 22, Table 5).

Stage V of development

In the 8 hens studied at Stage V of sexual development plasma LH levels continued to fall (mean, 2.8 ± 0.2 ng/ml) (Table 4). The LH response to progesterone (0.7 ± 0.1 ng/ml) was very small (Fig. 21, Table 5) and in 3 cases was not observed (e.g., hens 3237, 3238 Fig. 21). A mean maximal incremental change in plasma LH concentrations of 2.3 ± 0.2 ng/ml (n=5) was observed about 11 min after injection of LH-RH. This was significantly smaller ($P < 0.001$) than the response seen at Stage IV of sexual development (Fig. 22, Table 5). The time taken to achieve a maximal incremental change in LH levels was nearly twice that taken after injecting hens at Stages I and II with LH-RH (Table 5).

Stage VI of development

Four hens were examined at Stage VI of sexual development. The mean level of LH in the circulation had fallen further to 2.0 ± 0.2 ng/ml and was similar to that found in laying hens (Table 4). In contrast with birds at Stage V of development, plasma LH levels always rose after an injection of progesterone. After a mean latent period of 0.6 ± 0.1 h blood levels of the hormone rose slowly over a period of 2 to 3 h (Fig. 21). The mean maximal incremental change in LH levels

Table 5. Effect of 10 μg synthetic LH-RH/kg and 0.5 mg progesterone/kg on the secretion of LH during sexual maturation (means ± S.E.M.)

	LH response to i.v. injections of 10 μg LH-RH/kg			LH response to i.m. injections of 0.5 mg progesterone/kg				
†Stage of sexual maturation	No. of hens	Max. incr. change in plasma LH (ng/ml)	Time to maximum response (min)	No. of hens	Max. incr. change in plasma LH (ng/ml) *	Time to onset of response (h)	Time to maximum response (h) *	Rate of incr. change in plasma LH (ng/ml/h) *
I	9	23.5 ± 5.6	6.9 ± 0.7	9	No response	-	-	-
II	5	26.2 ± 7.7	6.4 ± 1.0	7	1.2 ± 0.1	2.5 ± 0.3	1.7 ± 0.3	0.8 ± 0.1
III	9	13.0 ± 1.1	8.4 ± 0.4	13	2.3 ± 0.2	1.7 ± 0.2	1.2 ± 0.1	2.3 ± 0.3
IV	6	5.1 ± 0.8	9.3 ± 1.6	12	1.2 ± 0.1	1.5 ± 0.1	2.0 ± 0.3	0.7 ± 0.1
V	5	2.3 ± 0.2	10.8 ± 1.4	5(8)‡	0.7 ± 0.1	1.1 ± 0.2	2.2 ± 0.7	0.4 ± 0.1
VI	2	2.1 ± 0.1	11.0 ± 1.0	4	1.0 ± 0.2	0.6 ± 0.1	2.2 ± 0.4	0.4 ± 0.1
VII	4	1.3 ± 0.1	13.5 ± 0.5	5	2.2 ± 0.6	0.3 ± 0.1	2.2 ± 0.6	1.2 ± 0.1

† see Table 4; * calculated from the point at which plasma LH levels started to rise.

‡ of the 8 hens injected with progesterone at Stage V, 3 showed no response and are not included in the calculations.

Figure 21

Changes in the concentration of plasma LH in individual hens after single intramuscular injections of 0.5 mg progesterone/kg at various stages of sexual maturation (see Table 4). ■——■ , LH concentration after injection (↑) of progesterone (P); ●·····● , LH concentration after injection of the progesterone vehicle. Vertical lines represent 95 % confidence limits; they are not shown for the responses to injections of vehicle. Ovarian and oviducal weights (g) are given for each hen.

Figure 22

Variation in the mean incremental changes in the concentration of plasma LH after single intravenous injections of 10 μg synthetic LH-RH/kg at the seven stages of sexual maturation (Roman numerals) shown in Table 4. There was no incremental change in LH concentration at any stage of sexual maturation in response to control injections of saline. Vertical lines represent ± S.E.M.

Fig. 2I

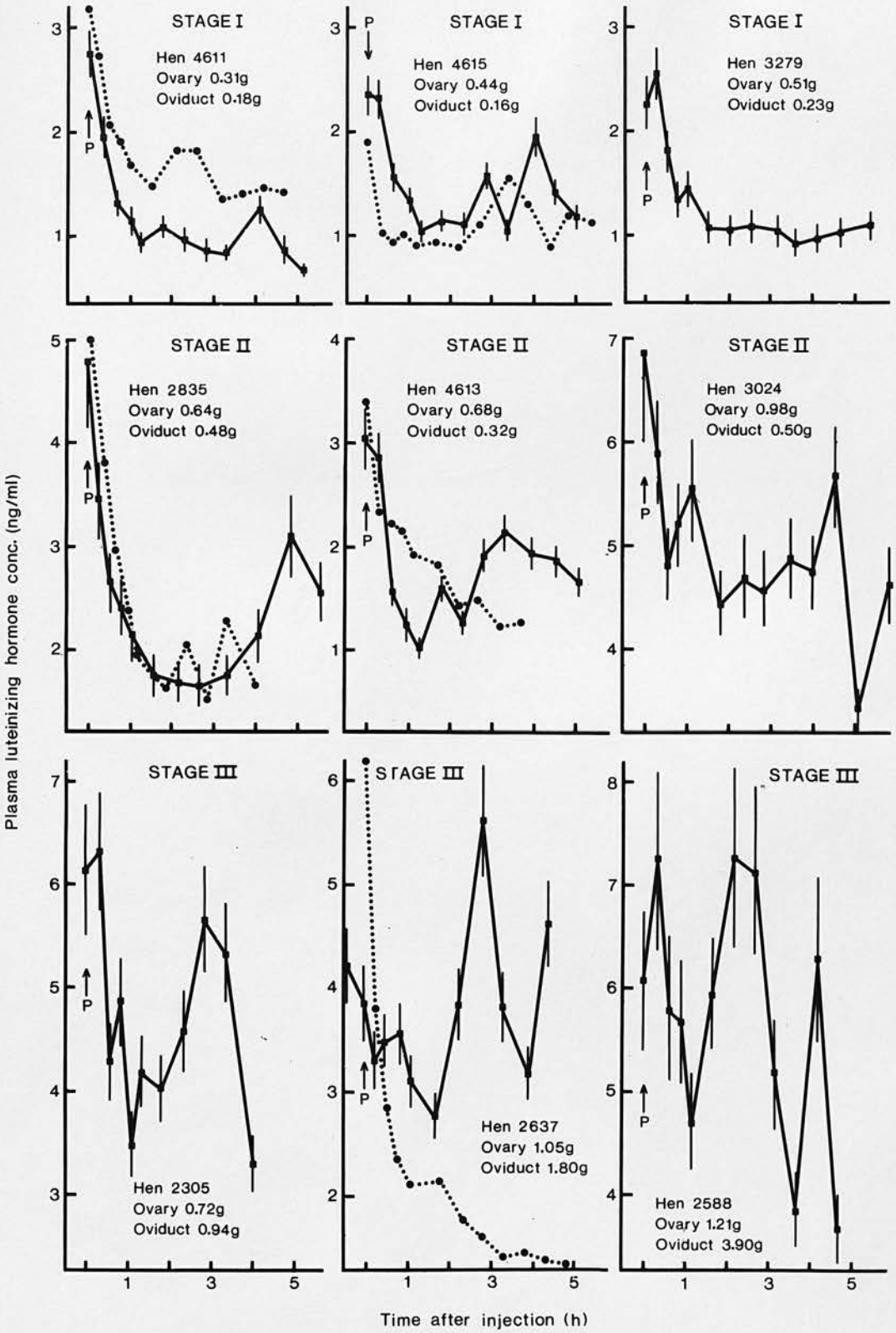

STAGE I
Hen 4611
Ovary 0.31g
Oviduct 0.18g
STAGE I
Hen 4615
Ovary 0.44g
Oviduct 0.16g
STAGE I
Hen 3279
Ovary 0.51g
Oviduct 0.23g
STAGE II
Hen 2835
Ovary 0.64g
Oviduct 0.48g
STAGE II
Hen 4613
Ovary 0.68g
Oviduct 0.32g
STAGE II
Hen 3024
Ovary 0.98g
Oviduct 0.50g
STAGE III
Hen 2305
Ovary 0.72g
Oviduct 0.94g
STAGE III
Hen 2637
Ovary 1.05g
Oviduct 1.80g
STAGE III
Hen 2588
Ovary 1.21g
Oviduct 3.90g
P
Plasma luteinizing hormone conc. (ng/ml)
Time after injection (h)

Fig. 21 (cont.)

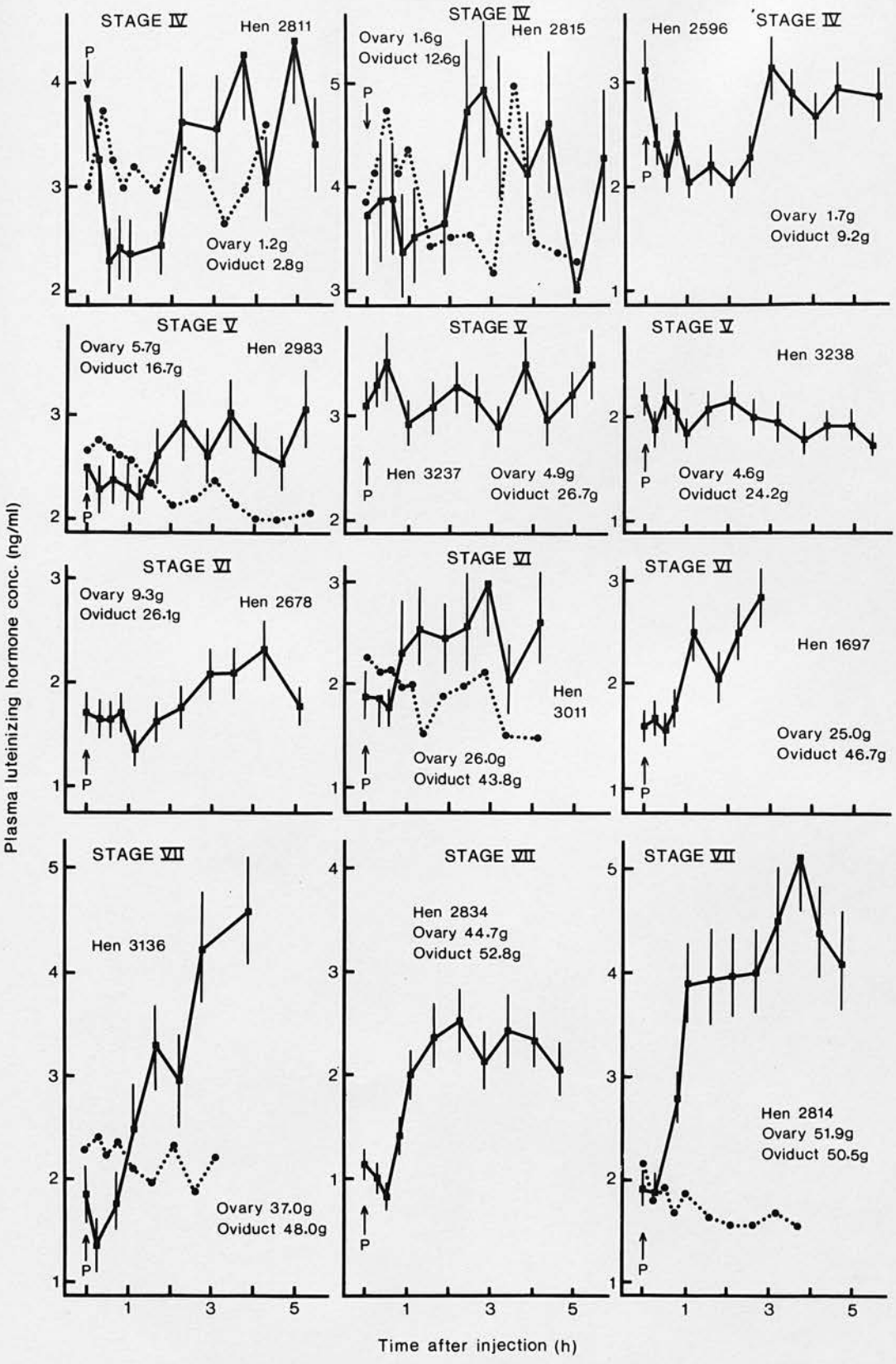
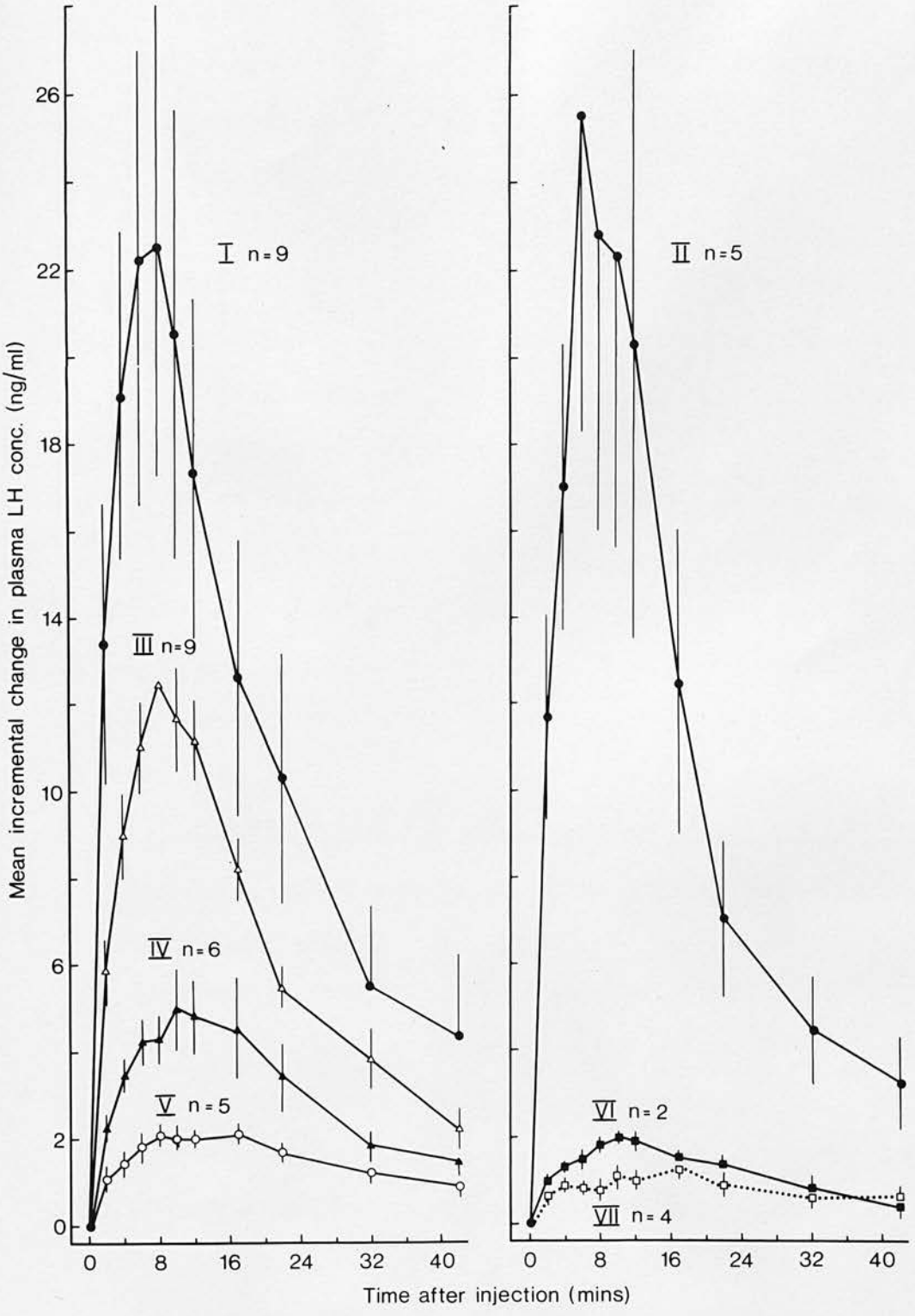

STAGE IV
Hen 2811
Ovary 1.2g
Oviduct 2.8g
STAGE IV
Ovary 1.6g
Oviduct 12.6g
Hen 2815
Hen 2596
STAGE IV
Ovary 1.7g
Oviduct 9.2g
STAGE V
Ovary 5.7g
Oviduct 16.7g
Hen 2983
STAGE V
Hen 3237
Ovary 4.9g
Oviduct 26.7g
STAGE V
Hen 3238
Ovary 4.6g
Oviduct 24.2g
STAGE VI
Ovary 9.3g
Oviduct 26.1g
Hen 2678
STAGE VI
Hen 3011
Ovary 26.0g
Oviduct 43.8g
STAGE VI
Hen 1697
Ovary 25.0g
Oviduct 46.7g
STAGE VII
Hen 3136
Ovary 37.0g
Oviduct 48.0g
STAGE VII
Hen 2834
Ovary 44.7g
Oviduct 52.8g
STAGE VII
Hen 2814
Ovary 51.9g
Oviduct 50.5g
P
Plasma luteinizing hormone conc. (ng/ml)
Time after injection (h)

Fig. 22


Mean incremental change in plasma LH conc. (ng/ml)
26
22
18
14
10
6
2
0
I n=9
II n=5
III n=9
IV n=6
V n=5
VI n=2
VII n=4
0
8
16
24
32
40
Time after injection (mins)

(2.1 ± 0.1 ng/ml (n=2)) after injections of LH-RH was similar to that observed at Stage V of sexual development (Table 5).

Stage VII of development

The 5 hens at Stage VII had already laid between 1 and 6 eggs when they were tested with progesterone and LH-RH. Mean plasma LH levels were 2.2 ± 0.2 ng/ml. The LH response to LH-RH was significantly less ($P < 0.01$) than that observed at Stage VI of development and resulted in a mean maximal incremental change in LH levels of 1.3 ± 0.1 ng/ml (Fig. 22; Table 5). On the other hand, the magnitude of the LH response to progesterone was greatly increased. Plasma LH levels started to rise 10 to 30 min after injection and reached a peak about 2.5 h later (Fig. 21). The mean maximal incremental change in LH levels was 2.2 ± 0.6 ng/ml (n=4) and was significantly greater ($P < 0.05$) than that observed at Stage VI of sexual development.

b. "Longitudinal" study of the effects of 10 µg synthetic LH-RH/kg and 0.5 mg progesterone/kg on plasma LH levels during sexual maturation.

Variation in response to progesterone and LH-RH in 8 out of 10 hens are shown in Fig. 23. In each bird, basal plasma LH levels started to rise 8 to 12 weeks before the onset of lay in conjunction with an increase in the rate of comb growth. A comparison with the "cross-sectional" study (Table 4) showed that this corresponded to the period when the ovary and oviduct started to develop. Levels of LH continued to rise for 3 to 4 weeks and then fell progressively until the first eggs were laid. By referring to Table 4 it was deduced that the ovaries must have been passing through Stages IV, V and VI, of

Figure 23

Longitudinal studies of changes in the concentration of plasma LH in response to single injections separated by an interval of 48 h of 10 μg synthetic LH-RH/kg (■) and 0.5 mg progesterone /kg (□) given at intervals of a fortnight during the 12 to 14 weeks before the onset of lay. Variations in plasma LH concentrations (●——●) and comb size (●·····●) during this period are also shown. The Roman numerals in the open boxes refer to the types of LH response to progesterone illustrated in Fig. 21. *, no response; ?, no observation was made. The numbers of eggs laid during the first six months of egg production are shown in parentheses after the hen number.

Fig. 23

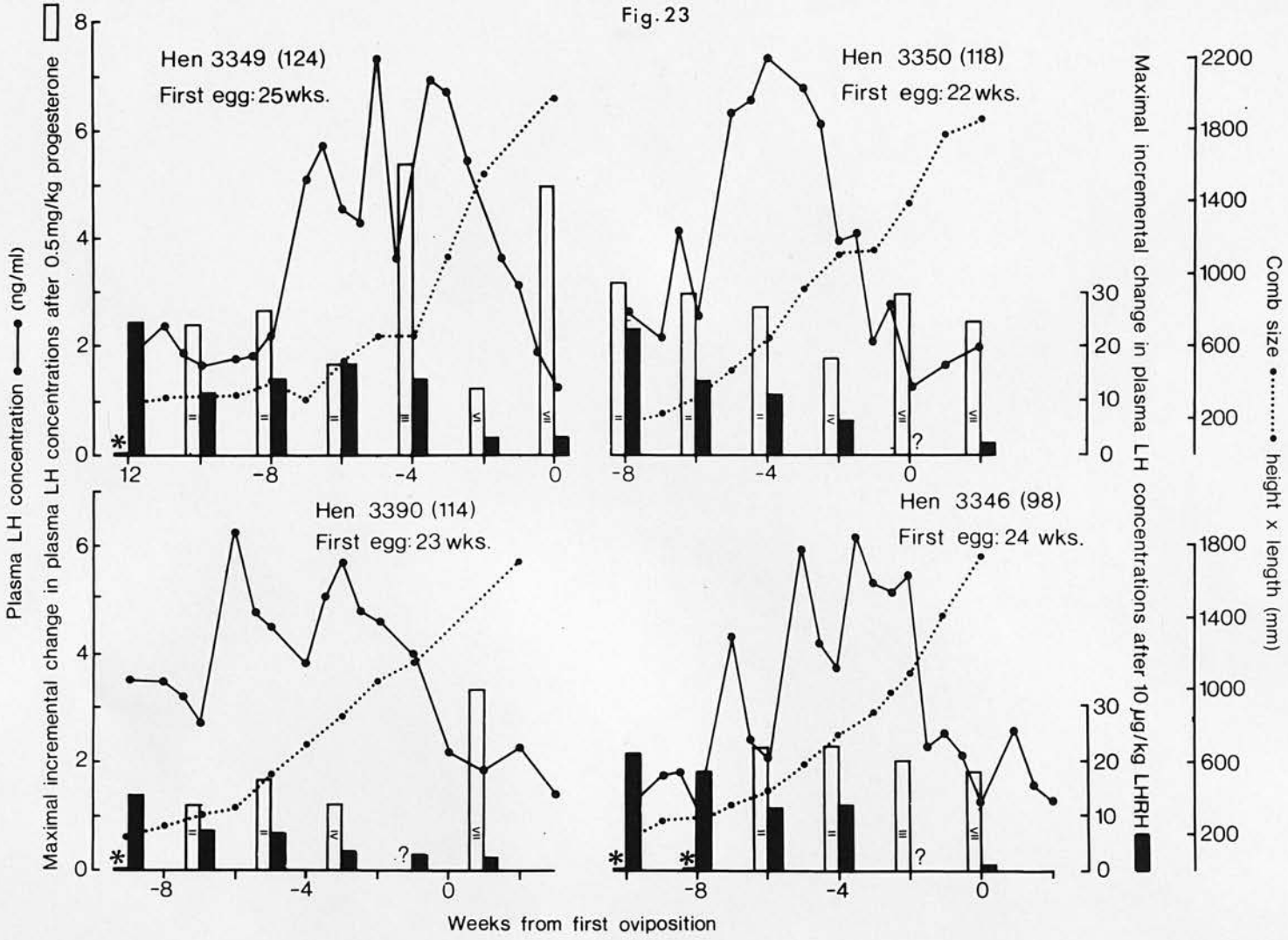
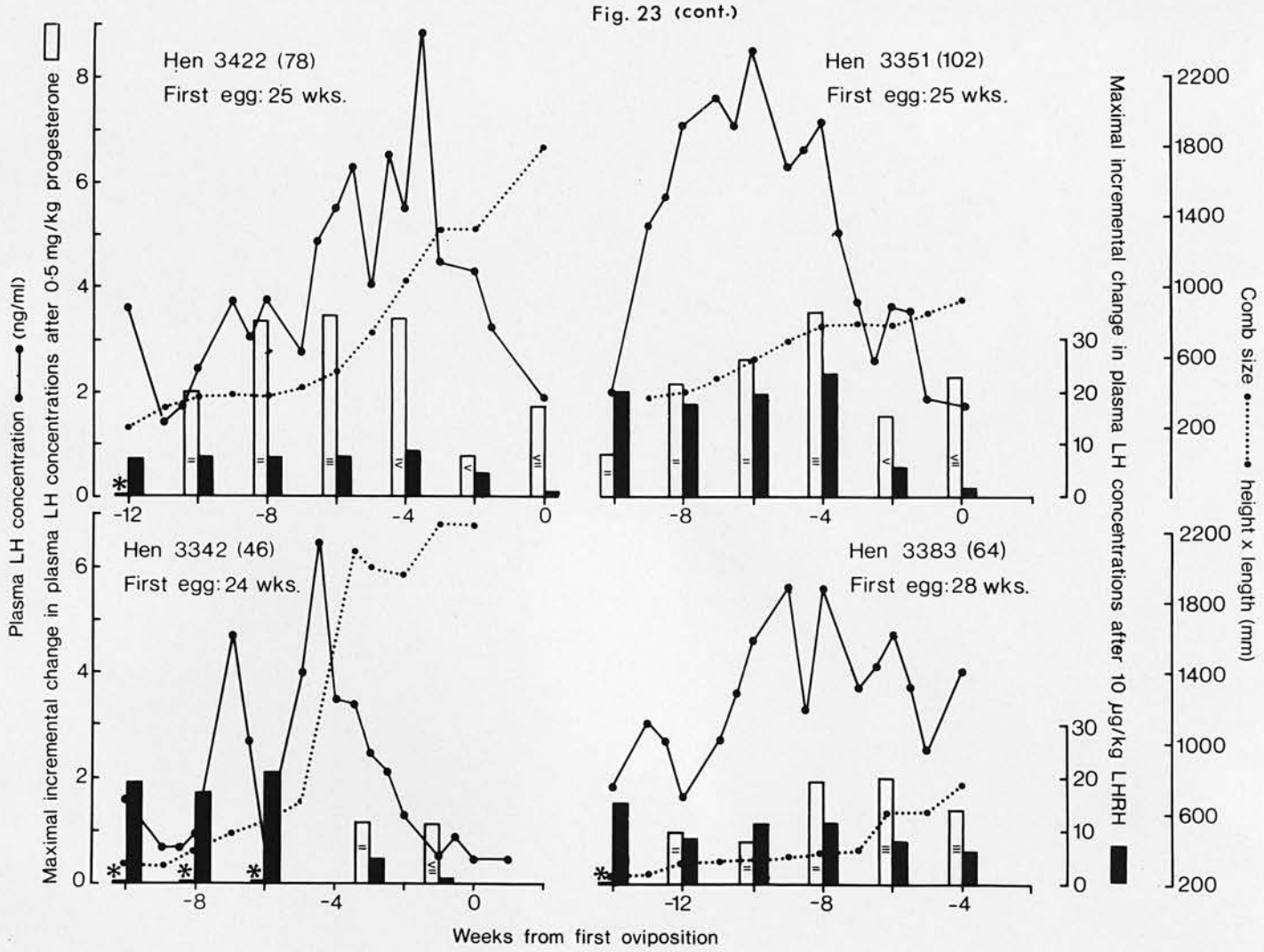

Hen 3349 (124)
First egg: 25 wks.
Hen 3350 (118)
First egg: 22 wks.
Hen 3390 (114)
First egg: 23 wks.
Hen 3346 (98)
First egg: 24 wks.
Plasma LH concentration ● (ng/ml)
Maximal incremental change in plasma LH concentrations after 0.5 mg/kg progesterone
Maximal incremental change in plasma LH concentrations after 10 µg/kg LHRH
Comb size ●·····● height x length (mm)
Weeks from first oviposition

Fig. 23 (cont.)


Hen 3422 (78)
First egg: 25 wks.
Hen 3351 (102)
First egg: 25 wks.
Hen 3342 (46)
First egg: 24 wks.
Hen 3383 (64)
First egg: 28 wks.
Plasma LH concentration (ng/ml)
Maximal incremental change in plasma LH concentrations after 0·5 mg/kg progesterone
Maximal incremental change in plasma LH concentrations after 10 µg/kg LHRH
Comb size height x length (mm)
Weeks from first oviposition

development during the period when plasma LH levels were falling. Throughout the period studied, body weights increased linearly; these are not shown in Fig. 23.

In agreement with the cross-sectional study, injections of progesterone were first found to stimulate the secretion of LH when basal levels of the hormone in the blood were rising. The type of response corresponded to that observed at Stage II of sexual development (Fig. 21). Subsequently, the types of LH response to progesterone followed the same sequence as that observed in the cross-sectional study (Fig. 23). Variations in the number of weeks during which a particular type of response to progesterone were observed in individual hens probably reflected differences in the rates of growth and development of their reproductive organs (cf. hens 3349, 3342, Fig. 23).

In contrast with the cross-sectional study, a reduced response to LH-RH was not consistently observed until prepubertal basal plasma LH levels were falling (Fig. 23).

c. Subsequent egg-laying performance of hens in relation to their LH responses to LH-RH and progesterone during sexual maturation.

The hens started to lay between 22 and 28 weeks of age (mean, 24 $\pm$ 0.6 weeks) and the number of eggs laid by each hen during the first 6 months ranged between 46 and 124 (mean, 93 $\pm$ 10 eggs). There was considerable variation in the magnitude and duration of the pre-pubertal LH peak, though the two best layers, hens 3349 and 3350 (Fig. 23) had larger pre-pubertal LH peaks and bigger pre-pubertal LH responses to progesterone than did the two poorest layers, hens

3342 and 3383 (Fig. 23). In hen 3342 which laid only 46 eggs during the first 6 months, progesterone injections failed to induce LH release until about 3 weeks before the first egg was laid. There was no relationship in individual hens between the number of eggs laid in the first 6 months of production and variations in the response of the pituitary to synthetic LH-RH during sexual maturation.

d. Sensitivity to the negative feedback effects of oestrogen in immature hens.

The increase in circulating levels of LH observed in pullets starting between 12 and 8 weeks before the onset of lay is consistent with the hypothesis (see page 4) that the onset of puberty is brought about by a rise in gonadotrophin levels resulting from a decrease in sensitivity of the negative feedback mechanism by which gonadal steroids regulate LH release. The previous study has demonstrated that the pre-pubertal rise in LH secretion is not associated with an increase in pituitary responsiveness to injections of synthetic LH-RH (page 81). The possibility that rising LH levels may have resulted from a decrease in sensitivity at the negative feedback centre of the hypothalamus was investigated by injecting pullets at various stages of sexual development with doses of between 0.1 and 2.0 mg oestradiol benzoate /kg and following the resulting changes in plasma LH concentration.

Hens which were either 14 to 17 weeks, 17 to 19 weeks or 21 weeks of age, or hens which had been laying for a few weeks were injected with either 0.1, 0.5, 1.0 or 2.0 mg oestradiol benzoate/kg, dissolved in arachis oil. At 09:00 h on the first day of the experiment, each hen was given a single injection of 0.3 to 0.5 ml arachis oil. LH levels were measured in plasma samples taken

immediately before the injection and at 6 and 12 h afterwards. At 09:00 h on the second day of the experiment each hen was given a single injection of oestradiol benzoate dissolved in the same volume of arachis oil. LH levels were measured in plasma samples taken at 6, 12, 24, 30, 36, 48, 54, 60, 72 and 78 h afterwards.

In hens of less than 21 weeks of age, plasma LH levels had fallen by between 20 and 50 % at 6 h after the injection of arachis oil on day 1. This was attributed to the stress of handling described on page 47. In some hens, LH levels were still depressed when oestradiol benzoate was injected on day 2, and consequently it was not possible to assess the negative feedback effects of the oestrogen injection. However, in hens of 21 weeks of age and in laying hens LH levels were not depressed after the injection of arachis oil and a comparison of the effects of oestrogen injections on plasma LH levels could be made between these two groups. In the laying hens, doses of 0.1, 0.5 or 1.0 mg oestradiol benzoate/kg caused no consistent change in plasma LH levels, while in the 4 hens injected at 21 weeks of age, doses of either 0.1, 0.5, 1.0 or 2.0 mg/kg caused a fall in LH concentration. The effects of the oestrogen injection were not immediate since the fall in LH levels was not observed 6 h after injection. However, at 12 h after the injection of single pullets with 0.1, 0.5, 1.0 or 2.0 mg oestradiol benzoate/kg, LH levels had fallen, respectively, to 95, 92, 87 and 85 % of the pre-injection value: 24 h after these injections, LH levels had fallen further to 72, 70, 67 and 51 %, respectively, of the pre-injection values. In hens injected with either 0.1 or 0.5 mg oestradiol benzoate/kg, LH levels then rose gradually to reach pre-injection values about 48 h later. However, in hens injected with

1.0 or 2.0 mg oestradiol benzoate/kg LH levels were maximally depressed to 47 and 67 %, respectively, of pre-injection values 48 h after injection. Pre-injection LH values were not regained until 78 h after oestrogen injection.

In conclusion, it was not possible to demonstrate any changes in sensitivity to the negative feedback effects of oestrogen during the pre-pubertal period when LH levels were rising. However, it was apparent that oestrogen injected into 21-week-old pullets, i.e. at a time when pre-pubertal LH levels were falling, exerts a stronger negative feedback effect on LH secretion than do injections in the laying hen.

e. Effects of priming injections of progesterone on the development of the positive feedback response.

While a positive feedback response to progesterone is observed in the immature hen at about 12 to 8 weeks prior to the onset of lay, the response does not mature fully until just a few days before the first egg is laid, at a time when the ovary contains large, rapidly-growing yellow yolky follicles. Since most of the progesterone present in the ovary may be produced by the large follicles (Furr, 1969; Shahabi, Norton & Nalbandov, 1975), the final priming of the positive feedback mechanism could depend on a changing ratio of progesterone to other steroids, notably oestrogen, in the circulation.

To test this possibility, 32 Thornber pullets of between 15 and 24 weeks of age were given a single priming injection of 0.5 mg progesterone/kg dissolved in arachis oil at 09:00 h on 3 successive days. On day 4, each hen was injected intramuscularly with 0.5 mg progesterone/kg dissolved in a propylene glycol solution (page 31).

Plasma LH concentration was measured in a blood sample taken by venepuncture at the time of the injection, and in samples taken at 15 or 30 min intervals (page 61) for a further 5 h. The pullets in this experiment were between Stages II and V of sexual maturation (Table 6).

At all stages of maturation, a single injection of 0.5 mg progesterone/kg on 3 successive days resulted in a fall in the basal level of plasma LH by between 33 and 53 % of the pre-priming concentration (Table 7). Mean values for selected parameters of the LH response to injected progesterone in the primed pullets (Table 7) were compared with those in unprimed pullets (Table 5).

Individual examples of the changes in plasma LH concentration following an intramuscular injection of 0.5 mg progesterone/kg into pullets primed with progesterone for 3 days previously are shown in Fig. 24. The concentration of plasma LH immediately before priming is shown in parentheses after the hen number. No significant difference, as indicated by the "Mann-Whitney" test, was observed in any of the selected parameters of the LH response to progesterone between unprimed and primed pullets at Stages II and III of sexual maturation. However, it was noticeable that 2 of the 10 progesterone-primed pullets at Stage II of maturation failed to show a positive feedback response in LH secretion to the injection of progesterone. The LH changes in one of these hens, number 4647, are shown in Fig. 24. Also, the incremental change in plasma LH concentration after injecting 8 primed pullets at Stage III with progesterone was very variable (Fig. 24), ranging from 0.48 to 3.21 ng/ml, and was reduced in magnitude in comparison to unprimed pullets (cf. Tables 7 and 5).

Table 6. Classification of stages of sexual maturation in progesterone-primed pullets

(means ± S.E.M.)

Stage of sexual development	No. of hens	Age (weeks)	Body wt (kg)	Comb size height x length (mm)	Ovarian (OV) & Oviducal (OD) wt (g)	Diameter of largest follicles (mm) and range	Pre-priming plasma LH (ng/ml)
II	10	16.6 ± 0.5	1.4 ± 0.1	481 ± 73	0.6 ± 0.0 (OV) 0.5 ± 0.1 (OD)	—	6.1 ± 0.7
III	8	21.5 ± 0.9	1.8 ± 0.1	516 ± 162	1.0 ± 0.1 (OV) 8.2 ± 2.3 (OD)	0.9 ± 0.5 (0-4)	7.7 ± 0.9
IV	6	22.7 ± 0.4	2.0 ± 0.1	933 ± 216	1.8 ± 0.2 (OV) 16.9 ± 3.3 (OD)	5.3 ± 0.3 (4-6)	3.3 ± 0.2
V	7	22.8 ± 0.4	1.9 ± 0.1	1151 ± 143	6.6 ± 1.2 (OV) 31.2 ± 2.7 (OD)	13.7 ± 1.6 (6-17)	3.1 ± 0.2

Justification for exclusion of 2 hens which did not respond.

Table 7. Effect of a single injection of 0.5 mg progesterone/kg on the secretion of LH in progesterone-primed pullets during sexual maturation (means ± S.E.M.)

†Stage of sexual maturation	No. of hens	Pre-priming plasma LH (ng/ml)	Post-priming plasma LH (ng/ml)	Time to onset of response (h)	Max. incr. change in plasma LH (ng/ml) *	Time to maximum response (h) *	Rate of incr. change in plasma LH (ng/ml/h) *
II	10‡	6.1 ± 0.7	3.2 ± 0.4	2.2 ± 0.2	1.4 ± 0.2	1.9 ± 0.2	0.9 ± 0.3
III	8	7.7 ± 0.9	3.7 ± 0.7	1.3 ± 0.2	1.8 ± 0.4	1.7 ± 0.3	1.1 ± 0.3
IV	6	3.3 ± 0.2	2.2 ± 0.2	0.7 ± 0.1	1.8 ± 0.3	2.1 ± 0.4	1.0 ± 0.3
V	7	3.1 ± 0.2	1.5 ± 0.2	0.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.2	1.1 ± 0.2

† see Table 6; * calculated from the point at which plasma LH levels started to rise.

‡ of the 10 hens injected with progesterone at Stage II, 2 showed no response and are not included in the calculations.

Figure 24

Changes in the concentration of plasma LH after a single intramuscular injection of individual progesterone-primed pullets with 0.5 mg progesterone/kg at Stages II to V of sexual maturation (see Table 6). Vertical lines represent 95 % confidence limits. Numbers in parentheses after the hen number indicate pre-priming plasma LH concentrations.
P, time of progesterone injection.

Fig. 24

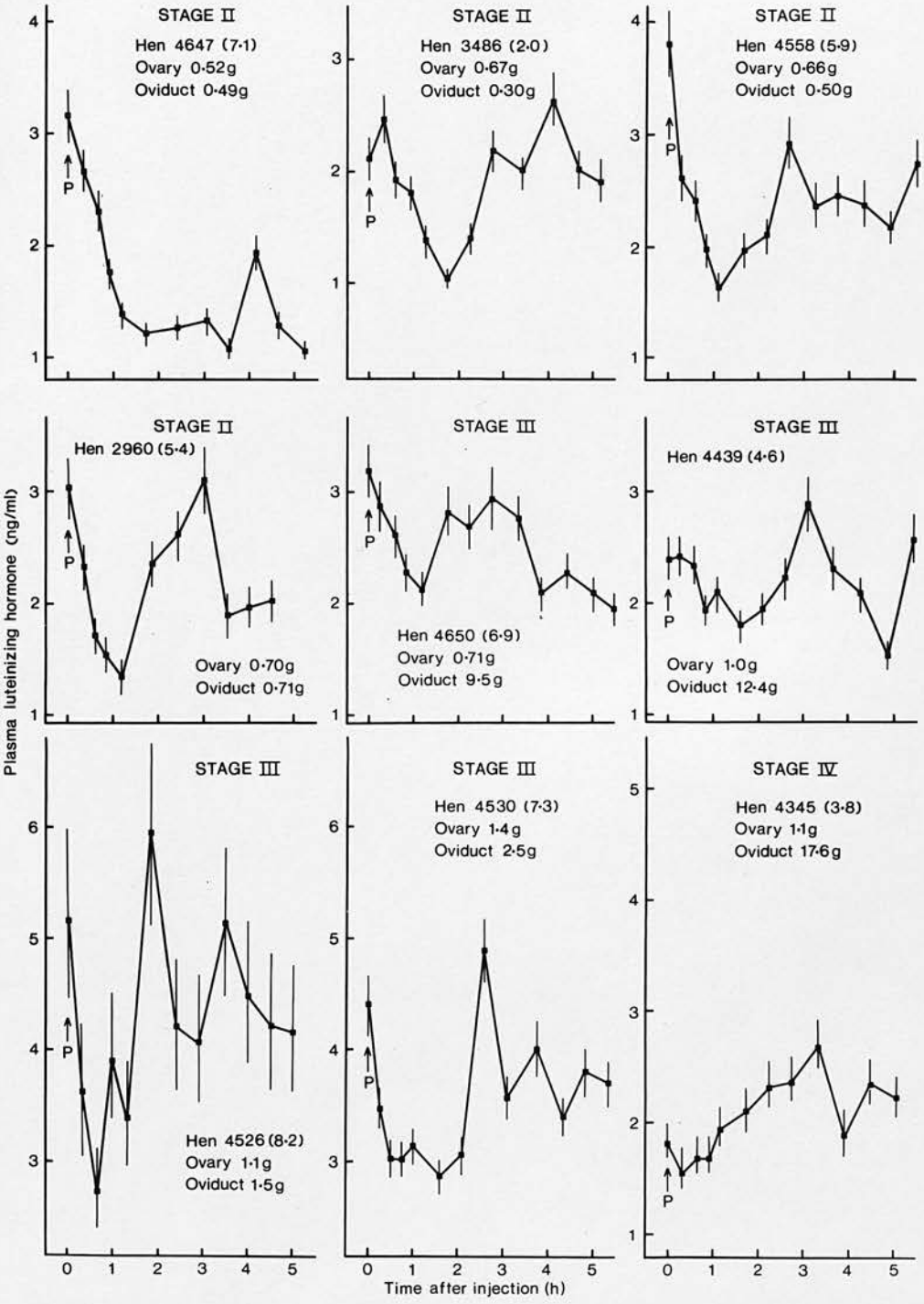
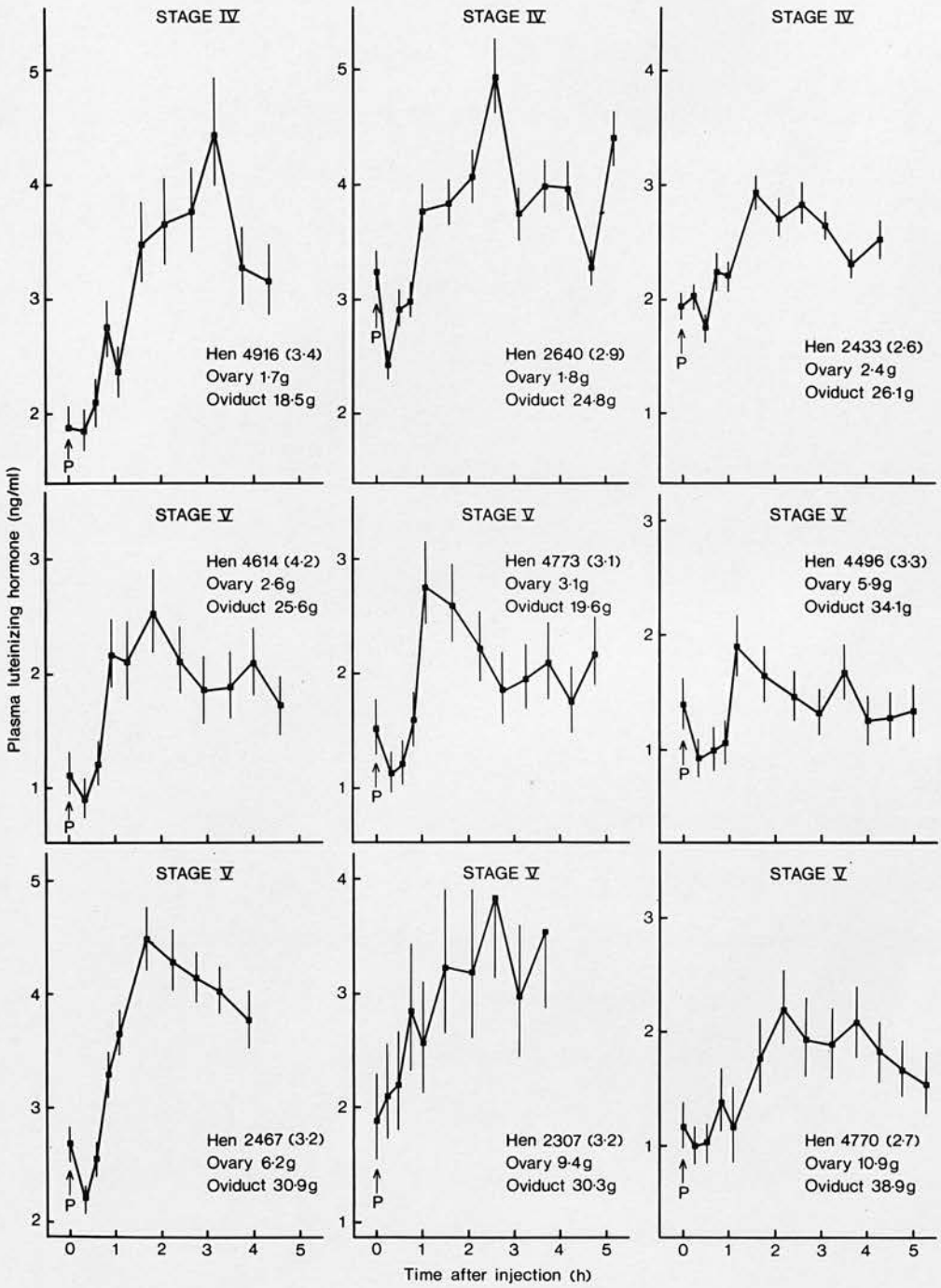

STAGE II
Hen 4647 (7·1)
Ovary 0·52g
Oviduct 0·49g
STAGE II
Hen 3486 (2·0)
Ovary 0·67g
Oviduct 0·30g
STAGE II
Hen 4558 (5·9)
Ovary 0·66g
Oviduct 0·50g
STAGE II
Hen 2960 (5·4)
Ovary 0·70g
Oviduct 0·71g
STAGE III
Hen 4650 (6·9)
Ovary 0·71g
Oviduct 9·5g
STAGE III
Hen 4439 (4·6)
Ovary 1·0g
Oviduct 12·4g
STAGE III
Hen 4526 (8·2)
Ovary 1·1g
Oviduct 1·5g
STAGE III
Hen 4530 (7·3)
Ovary 1·4g
Oviduct 2·5g
STAGE IV
Hen 4345 (3·8)
Ovary 1·1g
Oviduct 17·6g
P
Plasma luteinizing hormone (ng/ml)
Time after injection (h)

Fig. 24 (cont.)


STAGE IV
Hen 4916 (3·4)
Ovary 1·7g
Oviduct 18·5g
STAGE IV
Hen 2640 (2·9)
Ovary 1·8g
Oviduct 24·8g
STAGE IV
Hen 2433 (2·6)
Ovary 2·4g
Oviduct 26·1g
STAGE V
Hen 4614 (4·2)
Ovary 2·6g
Oviduct 25·6g
STAGE V
Hen 4773 (3·1)
Ovary 3·1g
Oviduct 19·6g
STAGE V
Hen 4496 (3·3)
Ovary 5·9g
Oviduct 34·1g
STAGE V
Hen 2467 (3·2)
Ovary 6·2g
Oviduct 30·9g
STAGE V
Hen 2307 (3·2)
Ovary 9·4g
Oviduct 30·3g
STAGE V
Hen 4770 (2·7)
Ovary 10·9g
Oviduct 38·9g
P
Plasma luteinizing hormone (ng/ml)
Time after injection (h)

This was associated with a significant reduction ($P < 0.05$) in the rate of incremental change in plasma LH levels compared with unprimed pullets.

At Stage IV of development, however, the maximal incremental change in plasma LH levels after injecting progesterone was generally greater than in the 6 unprimed pullets (mean, 1.8 ± 0.26 (S.E.M.) ng/ml ($n = 6$) versus 1.2 ± 0.1 ng/ml ($n = 6$))and the time to the onset of response was significantly ($P < 0.05$) reduced (0.7 ± 0.10 h versus 1.5 ± 0.1 h).

This difference in the response in LH secretion to injected progesterone between primed and unprimed pullets was even greater in the 7 pullets at Stage V of development. In progesterone-primed pullets, the final injection of progesterone in all cases resulted in a rise in plasma LH levels, the mean of which was 1.49 ± 0.21 ng/ml. This was significantly greater ($P < 0.05$) than the mean rise of 0.7 ± 0.1 ng/ml in 5 of 8 unprimed pullets which gave an LH response to progesterone when injected at Stage V of development. It is also greater than the mean maximal incremental change of 1.0 ± 0.2 ng/ml in the 4 unprimed hens at Stage VII. At Stage V of development there were significant differences ($P < 0.05$) between primed and unprimed pullets in time to onset of response (0.5 ± 0.06 h versus 1.1 ± 0.2 h)and the rate of incremental change in plasma LH concentration (1.1 ± 0.2 ng/ml/h versus 0.4 ± 0.1 ng/ml/h). There were no significant differences in the time to the maximum response.

Hence it appears that while progesterone-priming reduces basal plasma LH levels, it advances the maturation of the positive feedback response in LH secretion to a single injection of progesterone.

4. Maintenance of the positive feedback response

Since it was shown that the LH positive feedback response to progesterone only fully matured a few days before the onset of lay (page 82) at a time when the ovary contained large yolky follicles, it was of interest to see if the full response persisted in adult hens with ovaries which did not contain a full complement of yolky follicles. This involved attempting to induce an LH surge with a single injection of progesterone in a) hens which had begun to moult and stopped laying after about 1 year of egg production, and b) in regularly laying 1- to 2-year-old hens between 48 and 96 h after the surgical removal of their yolky follicles.

a. The positive feedback response in moulting (non-laying) hens

(i) Basal secretion of LH

Changes in plasma LH concentration were measured in individual hens during the period of moult until they restarted laying. Nine Shaver hens which had stopped laying for between 6 and 19 days were selected for this study. Blood samples were taken by venepuncture from each hen at intervals of 3 to 5 days until laying re-commenced.

Mean plasma LH levels were estimated from the total number of samples taken during 3 day periods preceding the first oviposition (Table 8). The mean plasma LH level in the first sample taken from 8 of the moulting hens which were between 11 and 38 days from the onset of lay (hen 8786 excluded) was 1.80 $\pm$ 0.19 (S.E.M.) ng/ml. No consistent rise or fall in mean LH levels was observed until about 10 days before lay. After this time they started to rise to reach a peak value of 2.87 $\pm$ 0.15 ng/ml (n = 9) at 5.5 $\pm$ 1.19 days before the first oviposition. Thereafter, plasma LH levels declined to about 1.64 $\pm$ 0.2

Table 8. Plasma LH levels in relation to the onset of lay in moulting hens (individual values and means ± S.E.M.)

Days before or after onset of lay	8786	540	422	9069	328	589	464	9818	226	Mean plasma LH (ng/ml) (n)
			2.10			1.24				1.64 ± 0.2 (4)
+3	1.82	1.42								
0	2.86	2.44	3.80	1.54	0.97	1.93			1.43	2.14 ± 0.4 (7)
-3		2.70		2.55	2.58	2.79	2.04	1.65	2.90	2.46 ± 0.2 (7)
-6	3.40	2.35	2.68			1.80	2.79	2.16		2.53 ± 0.2 (6)
-9		1.50	2.57	2.23	2.30	1.80			2.10	2.08 ± 0.1 (6)
-12			1.92		1.46		1.72	2.31		1.85 ± 0.2 (4)
-15			2.30	1.63	2.11	1.60	2.38		1.90	1.98 ± 0.1 (6)
-18			2.30	1.61			1.69	1.72	1.84	1.83 ± 0.1 (5)
-21				1.34	2.62			1.80	1.90	1.91 ± 0.3 (4)
-24				1.50	3.32		1.69	1.48	2.68	2.13 ± 0.4 (5)
-27					2.56		1.66	1.70		1.97 ± 0.3 (3)
-30				1.39	2.58		1.75		1.93	1.91 ± 0.2 (4)
-33				2.20			2.13	1.92	2.00	2.06 ± 0.1 (4)
-36				1.66	1.19			1.89		1.79 ± 0.2 (5)
-39							2.38	1.82		

ng/ml at which point laying re-commenced.

The mean LH concentration of 2.49 ± 0.13 ng/ml in blood samples taken between 3 and 8 days before oviposition was significantly greater ($P < 0.05$) than the concentration of 1.93 ± 0.10 ng/ml in samples taken between 12 and 17 days before oviposition.

(ii) Positive feedback response to progesterone

Seven Shaver hens which had stopped laying for 4 to 8 weeks were given a single injection of 0.5 mg progesterone/kg dissolved in a propylene glycol solution (page 31). Plasma LH levels were measured in a blood sample taken immediately before the injection and in 4 further samples taken at 1.5 h intervals thereafter. Each hen was then killed and the ovary and oviduct were dissected out and weighed. A maximal incremental change of 2.76 ng/ml was observed 1.5 h after progesterone was injected into one of these hens which was presumably on the point of lay since it had ovarian and oviducal weights of 58.7 g and 59.0 g respectively. Incremental changes of more than 0.73 ng/ml were not observed after progesterone injections in the 6 other hens, which had ovaries of between 3.3 and 33.4 g and oviducts of between 6.0 and 33.9 g, and were therefore not expected to start laying within the next few days. It therefore seems that the positive feedback system was not as responsive to progesterone in these birds as it was in the laying hen.

It is possible that the small response or lack of LH response to progesterone was due to a reduction in the secretion of steroids by the regressed or partly regressed ovary. This is suggested by the accompanying regression of the oviduct. Thus, steroids may be required to maintain the normal functioning of the positive feedback system.

b. Effect of removal of yolky follicles on the positive feedback response

The possibility that a fully developed ovary is necessary to maintain a mature positive feedback response was investigated in regularly laying hens by injecting them with progesterone at various times after surgically removing all the large yolky follicles.

(i) Basal secretion of LH

In an initial study the effects of removing follicles (partial ovariectomy) on the basal level of LH secretion were examined. A pre-operation blood sample was taken during the afternoon from 5 hens by means of venepuncture. The following day, all follicles larger than 5 mm diameter were removed from 4 of the hens by the method described on page 30 ; a control hen underwent a sham operation (page 30). Twenty-four hours after the operation, LH levels in the partially ovariectomized hens had fallen from a mean pre-operation value of 1.79 ± 0.41 (S.E.M.) ng/ml to 1.22 ± 0.20 ng/ml (n=4). In contrast, plasma LH levels in the sham-operated hen rose during the same period from 1.52 to 1.95 ng/ml. Between 24 and 72 h after the operation, LH levels in the 4 partially ovariectomized hens began to rise, and after 4 days had increased from 1.79 ± 0.41 ng/ml to 2.39 ± 0.26 ng/ml. LH levels either continued to rise further or remained elevated, and peak LH levels of 2.78 ± 0.33 ng/ml were observed 4.7 ± 0.55 days before the first oviposition. After peak LH levels were reached, plasma LH levels declined to pre-operation values at around the time of the first oviposition. The mean time taken for the ovaries to produce ovulable follicles was 11.2 ± 1.18 days.

No elevation in plasma LH concentration was observed in the sham-operated hen and it continued to lay normally throughout the experimental period.

(ii) Positive feedback response to progesterone

Fourteen Shaver hens laying regular sequences of more than 4 eggs were selected for this experiment. Each hen was given an injection of 0.5 mg progesterone/kg between 4 h after and 12 h before the time of a predicted ovulation. Blood samples were taken by venepuncture before and at 15 and 30 min intervals after injection (as described on page 61) for 3.5 h. A fortnight later, all yolky follicles of more than 5 mm diameter were removed from the ovaries of 11 of these hens by the method described on page 30 . A sham operation was performed on 3 other hens.

The LH response to progesterone was examined in each hen at intervals of either 48, 72 or 96 h after the operation. Blood samples were taken after progesterone injection at the same intervals as before, and immediately following completion of blood sampling each hen was killed and the ovary and oviduct were dissected out and weighed.

The maximal incremental change in LH levels in response to 0.5 mg progesterone/kg injected 48, 72 or 96 h after partial ovariectomy was variable but significantly reduced ($P < 0.01$ in all cases) in comparison with the response before the operation (Table 9). In contrast, in hens which underwent a sham operation, there was no difference between the LH response before and at 48 or 96 h after the operation (Table 9). It was concluded that the larger follicles of the ovary, and probably the steroids secreted by these follicles are

Table 9. Comparison of LH response to an injection of 0.5 mg progesterone/kg before and at 48, 72 & 96 h after the surgical removal of yolky ovarian follicles (means ± S.E.M. and range).

	Before operation		After operation			
No. of hens	Plasma LH (ng/ml)	Max. incr. change in plasma LH (ng/ml)	Plasma LH (ng/ml)	Max. incr. change in plasma LH (ng/ml)	Ovarian (OV) & Oviducal (OD) wt (g)	Interval after operation
4	2.3 ± 0.2	1.9 ± 0.5 (0.8 to 3.2)	0.9 ± 0.1	0.4 ± 0.2 (0.0 to 0.7)	7.3 ± 1.2 (OV) 45.8 ± 2.3 (OD)	48 h
*1	*1.7	*3.4	*2.2	*2.3	*27.4 (OV) *59.3 (OD)	
4	2.5 ± 0.3	2.0 ± 0.3 (1.4 to 2.6)	2.4 ± 0.2	0.7 ± 0.1 (0.6 to 0.9)	8.0 ± 0.8 (OV) 42.3 ± 0.2 (OD)	72 h
3	1.8 ± 0.1	3.0 ± 1.1 (1.2 to 5.1)	3.5 ± 0.5	1.1 ± 0.6 (0.2 to 2.4)	11.1 ± 0.5 (OV) 40.5 ± 0.7 (OD)	96 h
*2	*1.3, 2.4	*2.1, 1.5	*2.4, 1.6	*1.9, 2.1	*37.4, 44.0 (OV) *59.3, 56.8 (OD)	

*, sham-operated controls

Table 10. Effect of priming with gonadal steroids on the LH response to an injection of 0.5 mg progesterone/kg 96 h after the surgical removal of yolky ovarian follicles (means ± S.E.M. and range).

	Before operation		After operation			
No. of hens	Plasma LH (ng/ml)	Max. incr. change in plasma LH (ng/ml)	Plasma LH (ng/ml)	Max. incr. change in plasma LH (ng/ml)	Ovarian (OV) & Oviducal (OD) wt (g)	Priming treatment (/day for 3 days)
5	2.2 ± 0.3	2.4 ± 0.1	1.4 ± 0.2	0.6 ± 0.1 (0.2 to 1.1)	5.7 ± 1.3 (OV) 42.4 ± 2.5 (OD)	0.5 mg P/kg
5	2.0 ± 0.3	2.0 ± 0.2	2.5 ± 0.3	0.5 ± 0.2 (0.3 to 0.9)	13.8 ± 2.7 (OV) 35.2 ± 3.0 (OD)	0.1 mg OB/kg
6	1.9 ± 0.3	2.0 ± 0.2	1.1 ± 0.1	0.6 ± 0.3 (0.2 to 1.7)	5.3 ± 0.8 (OV) 41.8 ± 1.4 (OD)	0.5 mg P/kg + 0.1 mg OB/kg

P, progesterone; OB, oestradiol benzoate.

necessary to maintain a positive feedback response in the adult hen. Since LH levels only rose by a mean of 0.4 ± 0.2 ng/ml (n=4) in response to an injection of 0.5 mg progesterone/kg at 48 h after the operation, it appears that the positive feedback system requires constant or frequent priming by exposure to critical levels of sex steroids to keep it fully functional.

Effects of priming injections

An experiment was designed to investigate the effects of oestrogen and/or progesterone priming on the LH response in 16 Shaver hens to a single injection of 0.5 mg progesterone/kg given 96 h after the surgical removal of all yolky follicles larger than 5 mm diameter.

Starting on the day after the operation, 10 hens were given a single injection of either 0.5 mg progesterone/kg or 0.1 mg oestradiol benzoate/kg for 3 successive days; six other hens were given a single injection of both 0.5 mg progesterone/kg and 0.1 mg oestradiol benzoate /kg on 3 successive days. The steroids were dissolved in arachis oil. On the 4th day after the operation, all hens were injected with 0.5 mg progesterone/kg, in a solution of propylene glycol (page 31), and the LH levels were measured in plasma samples taken at 15 or 30 min intervals after the injection, as described on page 61 .

In contrast with the unprimed hens where basal LH levels rose by 1.64 ± 0.5 ng/ml from 1.83 ± 0.1 ng/ml to 3.46 ± 0.5 ng/ml (n=3) at 96 h after the operation, in the progesterone-primed and progesterone + oestrogen-primed hens, LH levels fell by 0.85 ± 0.2 ng/ml and 1.18 ± 0.5 ng/ml from pre-operation concentrations of 2.23 ± 0.3 ng/ml (n=5) and 1.87 ± 0.3 ng/ml (n=6) respectively (Table 10). However, LH levels in the oestrogen-primed hens rose by a mean of 0.48 ± 0.2 ng/ml

from a pre-operation concentration of 2.03 ± 0.3 ng/ml (n=5), which was significantly different from the changes in LH concentration after either progesterone priming ($P < 0.01$) or progesterone + oestrogen priming ($P < 0.02$). It was noticeable that the ovarian weight in the oestrogen-primed hens was significantly greater than in the progesterone-primed ($P < 0.05$) or in the progesterone + oestrogen-primed ($P < 0.01$) hens (Table 10). Also the ovarian weights of the progesterone-primed and progesterone + oestrogen-primed hens were lower than in unprimed hens 96 h after the operation. It is possible that the priming with oestradiol benzoate and progesterone or with progesterone alone reduced the rate of follicular growth. It is more probable, however, that this reflects differences between the primed and unprimed groups of hens in the amount of ovarian tissue removed during the operation.

Although the maximal incremental change in LH levels after priming with steroids was variable, none of the priming treatments restored the positive feedback response to progesterone (Table 10).

Conclusion

It is concluded that the mature positive feedback mechanism requires constant or frequent priming by steroids such as are secreted from the ovary of the laying hen. When the composition and concentration of steroidal secretion is altered by surgically removing the large ovarian follicles, the positive feedback response is abolished and the priming schedule used in this experiment was unable to restore the response.

5. Role of steroids in the control of oviposition

While examining the effects of injections of progesterone or

testosterone on LH secretion in the laying hen it was observed that often the oviposition of the egg in the oviduct at the time of injection was delayed by several hours or days. This was further investigated.

Shaver hens, laying regular sequences of between 3 and 10 eggs, were given a single injection of either progesterone, oestradiol-17β, oestrone, androstenedione or testosterone dissolved in a solution of propylene glycol (page 31) at various times in relation to the occurrence of an oviposition. The exact times of oviposition were recorded automatically (see page 27).

For the purpose of the calculations in this study, an oviposition was considered to occur simultaneously with the succeeding ovulation, except at the beginning and end of a sequence when these events do not occur together. The first ovulation in a sequence was estimated to occur at approximately 05:00 h (see page 62). All advances or delays in the time of oviposition were calculated to the nearest 0.5 h.

Observations on the effects of steroids on oviposition were made in conjunction with the experiments involving a determination of the effects of these steroids on LH secretion (see pages 58-71). However, a more detailed examination of the effects of progesterone on oviposition involved the use of data taken from experiments other than those shown on pages 58 to 71.

a. Progesterone

When hens with oviducal eggs were injected with progesterone at various stages of the ovulatory cycle, oviposition of these eggs was frequently advanced or delayed. Neither the occurrence nor time of ovulation or oviposition was affected in 8 control hens injected with

the vehicle for progesterone, i.e. a solution of propylene glycol (page 31).

(i) Progesterone injections given 0 to 6 h after ovulation (27 to 21 h before a predicted ovulation)

In 47 of 55 hens injected with 0.5 mg progesterone/kg between 0 and 6 h after an ovulation, oviposition of the resulting egg was delayed by 1.25 to 11.0 h (mean, 7.00 ± 0.34 h) beyond the time predicted from examination of the oviposition times of previous sequences (Effect A, Fig. 25b; Tables 11 and 12a). The injection determined the time at which the oviposition occurred since the interval between these two events was fairly constant, ranging between 28.0 and 33.0 h (mean, 30.6 ± 0.22 h). The delayed oviposition became the last of the sequence because on the 2nd day after injection no egg was laid. The next egg was laid early on the 3rd day following progesterone injection and commenced a new sequence (Fig. 25b). Subsequent ovipositions were resynchronized so that they continued according to the established sequence for the hen (e.g. hens 4197 and 4146, Table 12a). In the remaining 8 hens injected with 0.5 mg progesterone/kg oviposition was delayed by between 14.5 and 20.5 h (mean, 17.6 ± 1.04 h) until early on the 2nd day after injection (Table 11). Such an effect was more commonly seen after injecting progesterone between 6 and 15 h after ovulation, and is described in more detail in the next section (Effect B, Fig. 25c; Table 11).

Because of the way in which a progesterone injection disrupted a sequence of ovipositions (Fig. 25b), it was reasonable to believe that the steroid interfered with the development or integrity of the

ovarian follicle next due to ovulate. To investigate this further, 20 hens were injected with 0.5 mg progesterone/kg between 0 and 6 h after an ovulation and were killed in the morning, 48 h later, in order to examine their ovaries and oviducts. This interval of 48 h was estimated to allow sufficient time for the occurrence of both a delayed oviposition and the next ovulation. At autopsy there were no signs of atresia amongst the large yolky follicles in any of the 20 hens and, with the exception of 4 cases, each hen had a membranous egg in either the magnum or the shell gland of the oviduct. An identical situation was observed in 8 control hens autopsied at the same time of day following the first ovulation of a sequence. The mean weights of the most recent post-ovulatory follicles in the control (0.53 $\pm$ 0.02 g) and experimental hens (0.55 $\pm$ 0.02 g) were not significantly different. These observations show that after an injection of progesterone ovulation of the largest follicle was delayed until the normal time for the first ovulation of a sequence (Fig. 25b). In 4 of the 20 hens a hard-shelled egg was found in the oviduct at autopsy and the mean weight of the largest post-ovulatory follicle was 0.37 $\pm$ 0.01 g. This suggested that there had not been a recent ovulation. In view of the absence of follicular atresia, the progesterone injection given 2 days previously must still have been exerting a delaying effect on the ovulation of the largest ovarian follicle.

When 12 hens were injected with 0.1 mg progesterone/kg, ovipositions were delayed for a few hours in all but two cases (Table 11). Ovulations were also delayed and sequences terminated (Effect A, Fig. 25b). The interval between injection and oviposition was

significantly shorter ($P < 0.001$) than after giving 0.5 mg progesterone /kg (25.0 $\pm$ 0.35 h versus 30.6 $\pm$ 0.22 h).

Injections of 0.05 mg progesterone/kg delayed oviposition and terminated the sequence in 4 of 10 hens (Table 11); the remaining 6 continued laying without disruption.

(ii) Progesterone injections given 6 to 15 h after ovulation (21 to 12 h before a predicted ovulation)

In 16 of 37 hens injected with 0.5 or 0.1 mg progesterone/kg between 6 and 15 h after ovulation, oviposition of the resulting egg was delayed by between 15.5 and 28.0 h (mean, 22.00 $\pm$ 1.08 h) and occurred early on the 2nd day after injection (Effect B, Fig. 25c; Table 12b, hens 4163 and 4226). In 2 other hens oviposition was delayed until early on the 3rd day after injection (Effect D, Table 11; Table 12, hens 4127 and 4220). Examination of the oviposition records of hens showing Effects B and D suggested that after an injection of progesterone, the next ovulation was delayed until early on the 2nd day (Effect B, Fig. 25c) or the 3rd day (Effect D) after the injection, and at about the same time as a delayed oviposition. It was therefore evident that the timing of the delayed oviposition was related to that of the next ovulation rather than to the time of injection.

In 4 of the 37 hens the delayed oviposition occurred late on the 2nd day after progesterone injection and judging from the subsequent laying records did not occur simultaneously with an ovulation (Effect C, Tables 11 and 12b). In one case (hen 4224, Table 12b) it appeared that the first ovulation after injection occurred about 10 h before the oviducal egg was expelled.

In a further 4 hens the time of oviposition was related to that of the injection, which resulted in Effect A (Fig. 25b, Tables 11 and 12a).

The laying patterns were not affected at all in 11 of the 37 hens (Table 11).

In 5 of 9 hens injected with 0.05 mg progesterone/kg oviposition appeared to be delayed, resulting in Effect A (Fig. 25b). The injections did not disrupt laying patterns in the remaining 4 hens.

(iii) Progesterone injections given 15 to 18 h after ovulation (12 to 9 h before a predicted ovulation).

In all but 1 of 16 hens injected with 0.1, 0.5 or 0.05 mg progesterone/kg between 12 and 9 h before a predicted ovulation, the oviducal egg was expelled prematurely by between 1.5 and 4.25 h (Table 11). The interval between injection and induced oviposition ranged between 6.0 and 10.0 h (Table 11). Examination of the oviposition records for 9 of these hens showed that the injection caused the next oviposition about 2.5 h earlier than predicted. This suggested that the steroid advanced ovulation by the same period (Effect E, Fig. 25d; Tables 11 and 12c). Subsequent ovipositions then occurred as in a normal sequence (e.g. hens 4148 and 4242, Table 12c). In the remaining 6 hens the injection probably blocked the impending ovulation (Effect F, Tables 11 and 12c).

(iv) Progesterone injections given 18 to 27 h after ovulation (9 to 0 h before a predicted ovulation)

It has been shown that during the 9 h prior to ovulation there is a pre-ovulatory surge of ovarian steroids and LH (page 23). In 37 hens injected with progesterone during this period the impending

Table 11

Effects of a single injection of progesterone at various stages of the ovulatory cycle on the timing of oviposition ± S.E.M.

Dose (mg/kg)	Intervals (h) between injection and last ovulation and next predicted ovulation (in brackets)	Interval (h) between injection and oviposition of egg in oviduct	Type of effect†	Advance (-) or delay (+) in oviposition of egg in oviduct (h)	Advance (-) or delay (+) in oviposition of egg next ovulating (h)
0.5	0 - 6 (27 - 21)	30.6 ± 0.22 (47)*	A	+ 7.0 ± 0.34	Variable
		40.0 ± 1.71 (8)	B	+17.6 ± 1.04	
	6 - 15 (21 - 12)	18.8 ± 1.07 (8)	/	no effect	
		31.0 ± 0.87 (3)	A	+10.4 ± 1.66	
		39.4 ± 1.25 (14)	B	+22.6 ± 1.10	
		49.0 ± 0.67 (4)	C	+31.7 ± 0.84	
		63.0, 67.0 (2)	D	+45.5 ++50.0	
	15 - 18 (12 - 9)	7.0 ± 0.52 (5)	E	- 3.0 ± 0.45	-2.4 ± 0.16 (3)*
			F		Blocked (2)
	18 - 21 (9 - 6)	6.9 ± 0.17 (5)	/	no effect	no effect (5)
	21 - 27 (6 - 0)	3.1 ± 0.37 (21)	G	no effect	no effect (9)
					+2.9 ± 0.37 (12)
0.1	0 - 6 (27 - 21)	22.0, 21.0 (2)	/	no effect	Variable
		26.0 ± 0.35 (10)	A	+ 3.4 ± 0.53	
	6 - 15 (21 - 12)	18.5 ± 1.04 (3)	/	no effect	
		27.0 (1)	A	+ 9.5	
		35.5, 40.0 (2)	B	+15.5, 22.0	
	15 - 18 (12 - 9)	8.5 ± 0.70 (5)	E	- 2.5 ± 0.22	Blocked (3)
			F		-2.0, -3.0 (2)
	18 - 27 (9 - 0)	3.0 ± 1.27 (6)	/	no effect	no effect
0.05	0 - 6 (27 - 21)	24.0 ± 1.00 (6)	/	no effect	Variable
		25.2 ± 0.42 (4)	A	+ 3.0 ± 1.04	
	6 - 15 (21 - 12)	15.4 ± 1.58 (4)	/	no effect	
		21.0 ± 0.59 (5)	A	+ 4.7 ± 1.40	
	15 - 18 (12 - 9)	11.5 (1)	/	no effect	no effect
		9.0 ± 0.61 (5)	E	- 3.0 ± 0.23	-3.0 ± 0.29 (4)
					Blocked (1)
	18 - 21 (9 - 6)	7.5 ± 0.36 (5)	/	no effect	no effect

† see Table 13 and Fig. 26

* number of hens

Table 12

Examples of times of oviposition in hens with sequences disrupted by injections of 0.5 mg progesterone/kg given at various times during the ovulatory cycle; lights on at 07:00 h and off at 21:00 h.

(a) Injections 0 - 6 h after ovulation

Effect A[†]

Hen 4197. Interval between injection and oviposition, 28:37 h; delay approx. 8.0 h

08:22 10:36 12:20 16:35 * 07:18 17:47 * 08:37 10:31 13:16 16:53 *
13:10

Hen 4146. Interval between injection and oviposition, 29:29 h; delay approx. 9.5 h

07:54 10:07 12:25 16:02 * 08:01 19:41 * 07:17 09:03 12:03 16:00 *
14:12

(b) Injections 6 - 15 h after ovulation

Effect B[†]

Hen 4163. Interval between injection and oviposition, 36:32 h; delay approx. 20.0 h

09:16 12:45 17:24 * 08:39 * 07:54 11:37 16:58 *
19:22

Hen 4226. Interval between injection and oviposition, 41:00 h; delay approx. 22.0 h

07:51 09:11 10:38 12:48 15:53 * 07:23 * 07:00 08:28 09:49 11:14 13:48 16:00
14:00

Effect C

Hen 4224. Interval between injection and oviposition, 48:47 h; delay approx. 33.0 h

08:37 10:56 12:15 16:27 * 08:37 * 20:07 11:43 * 08:42 11:05 13:18 17:14 *
19:20

Hen 4133. Interval between injection and oviposition, 49:31 h; delay approx. 32.5 h

07:58 09:13 10:15 10:30 * 20:43 * 09:20 12:16 16:33 *
19:12

Effect D

Hen 4217. Interval between injection and oviposition, 64:15 h; delay approx. 47.5 h

07:40 08:29 08:51 08:33 09:35 11:00 14:25 * 07:40 08:51 09:08 * * 09:03
16:48

09:31 09:21 09:54 09:58 10:04 10:13 10:44 17:54 *

Hen 4220. Interval between injection and oviposition, 55:52 h; delay approx. 45.0 h

07:32 09:14 10:51 12:40 16:19 * 07:42 * * 06:47 08:02 09:34 12:32 16:28 *
22:55

Table 12 contd.

(c) Injections 12 - 9 h before ovulation

Effect E†

Hen 4148. Interval between injection and oviposition, 06:01 h; advance approx. 3.0 h

08:11 10:29 12:13 15:36 * 04:43 08:12 10:24 12:57 14:33 *

22:42

Hen 4242. Interval between injection and oviposition, 06:50 h; advance approx. 4.0 h

07:19 09:04 10:32 12:39 16:17 * 07:17 04:42 07:44 09:32 11:21 13:22 16:34

21:52

Effect F

Hen 4160. Interval between injection and oviposition, 06:30 h; advance approx. 4.0 h

07:43 10:00 12:18 16:20 * 07:54 05:27 * 12:14 16:58 *

22:57

Hen 4145. Interval between injection and oviposition, 07:57 h; advance approx. 3.0 h

08:47 10:57 14:03 17:14 * 08:01 06:49 * * 11:00 12:17 16:04 *

22:52

(d) Injections 9 - 0 h before ovulation

Effect G

Hen 4150. Interval between injection and oviposition of egg next ovulating, 34:12 h; delay approx. 2.0 h

08:25 11:12 14:42 18:03 * 08:22 10:46 16:50 *

06:38

Hen 4143. Interval between injection and oviposition of egg next ovulating, 30:21 h; delay approx. 2.0 h

07:51 09:23 10:04 11:26 12:45 13:31 15:50 * 06:30 08:06 12:45 13:32 16:27

06:24

(e) Injections 6 - 0 h before predicted terminal oviposition of sequence

Hen 4116. Interval between injection and oviposition of induced ovulation, 32:11 h

09:04 12:05 13:38 16:11 * 09:35 10:57 12:24 15:48 21:59 * 09:30

13:48

Hen 4230. Interval between injection and oviposition of induced ovulation, 32:51 h

07:42 09:55 11:01 14:34 * 07:36 09:38 11:33 12:41 14:23 18:18 * 07:28

09:27

†, see Fig. 26 ; ↑ , time of progesterone injection; *, day of no oviposition

Figure 25

A diagrammatic representation of alterations in the temporal relationship between oviposition and ovulation after injections of progesterone at various stages of the ovulatory cycle (c.f. Tables 11 and 12). The black horizontal bars represent the hours of darkness; the dotted lines indicate expected events if progesterone had not been injected; ^ represents a pre-ovulatory surge of progesterone; P, time of progesterone injection; OVP, oviposition; OVL, ovulation.

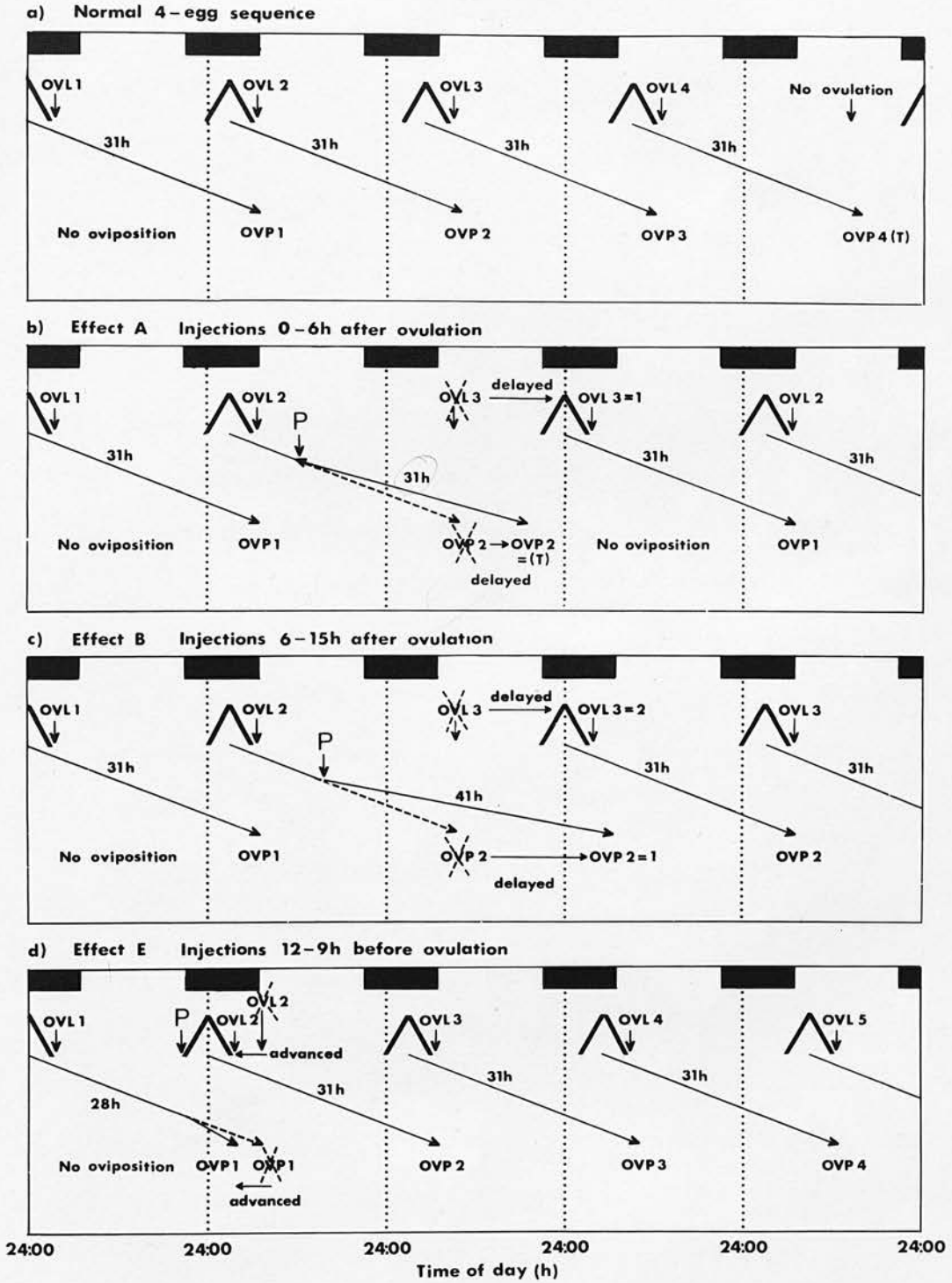
a) a normal 4-egg sequence.

b) Effect A, chiefly observed after injecting 0.1 or 0.5 mg progesterone/kg at 0 to 6 h after ovulation. Oviposition is delayed and does not occur at the same time as an ovulation. The next ovulation is delayed and becomes the first of a new sequence.

c) Effect B, chiefly observed after injecting 0.5 mg progesterone/kg at 6 to 15 h after ovulation. Oviposition is delayed and becomes the first of a new sequence. Ovulation is also delayed and occurs at the same time as the delayed oviposition.

d) Effect E, observed after injecting 0.05, 0.1 or 0.5 mg progesterone/kg at 12 to 9 h before ovulation. Ovulation and oviposition are advanced by about 3 h.

Fig. 25


a) Normal 4-egg sequence
OVL 1
OVL 2
OVL 3
OVL 4
No ovulation
31h
31h
31h
31h
No oviposition
OVP 1
OVP 2
OVP 3
OVP 4 (T)
b) Effect A Injections 0-6h after ovulation
OVL 1
OVL 2
OVL 3
delayed
OVL 3=1
OVL 2
P
31h
31h
31h
31h
No oviposition
OVP 1
OVP 2
OVP 2 =(T)
delayed
No oviposition
OVP 1
c) Effect B Injections 6-15h after ovulation
OVL 1
OVL 2
OVL 3
delayed
OVL 3=2
OVL 3
P
31h
31h
31h
41h
No oviposition
OVP 1
OVP 2
OVP 2=1
delayed
OVP 2
d) Effect E Injections 12-9h before ovulation
OVL 1
P
OVL 2
OVL 2
advanced
OVL 3
OVL 4
OVL 5
31h
31h
31h
28h
No oviposition
OVP 1
OVP 1
advanced
OVP 2
OVP 3
OVP 4
24:00
24:00
24:00
24:00
24:00
24:00
Time of day (h)

oviposition occurred at the predicted time. However, in 12 of 21 hens injected less than 6 h before an oviposition with 0.5 mg progesterone/kg the oviposition of the next egg was delayed by about 3 h (Effect G, Tables 11 and 12d).

(v) Progesterone injections given 6 to 0 h prior to the terminal oviposition of a sequence (18 to 27 h after ovulation, i.e. approximately 24 to 15 h before the next predicted ovulation)

Injections of either 0.5 (18 hens), 0.1 (2 hens) or 0.05 (2 hens) mg progesterone/kg given 6 to 0 h before the last oviposition of a sequence did not prevent it from occurring at the predicted time. However, an injection invariably induced premature ovulation (Table 12e). The interval between injection and oviposition ranged from 31.0 to 38.25 h (mean, 34.0 $\pm$ 0.57 h). An induced oviposition extended a sequence by one egg, thereby delaying the start of a new sequence by a day.

b. Deoxycorticosterone (DOC)

The effects of deoxycorticosterone acetate injections on oviposition in hens with oviducal eggs were not as consistent as those observed after the injection of progesterone. Seven hens were injected with either 0.5 or 1.0 mg DOCA/kg between 0 and 6 h after a predicted ovulation. In 2 hens, oviposition of this egg was delayed by 3 and 12 h and in both cases occurred at 32.5 h after the injection (Effect A; see Fig. 25b). This terminated the sequence presumably by delaying the next ovulation since no egg was laid on the second day after the injection. In 2 other hens, oviposition was delayed by 20.5 and 22.0 h and occurred early on the second day after injection at about the time

of the next ovulation (Effect B; see Fig. 25c). It appears that this ovulation had been delayed as in Effect A. In 2 hens, the oviducal egg was laid early on the 3rd day after the injection, following a delay of 67.0 and 69.0 h (Effect D), while the remaining 1 hen was unaffected. The times of oviposition occurring after the injection of 0.1 mg DOCA/kg in 6 hens (page 68) were not recorded due to a fault in the automatic recording devices.

Seven hens were injected with either 0.5 or 1.0 mg DOCA/kg between 6 and 10 h after a predicted ovulation. In 5 cases, oviposition of this egg was delayed by a mean of 22.4 $\pm$ 0.55 h until early on the 2nd day following the injection (Effect B). In the other 2 hens, oviposition was delayed by 10.5 and 5.0 h and occurred at 28.0 and 32.5 h after the injection (Effect A).

Injection of 4 hens with 0.5 or 1.0 mg DOCA/kg at 4 to 0 h before the terminal oviposition of a sequence caused premature ovulation in only 1 case. In this bird DOCA caused plasma LH levels to rise by 1.3 ng/ml from 1.05 ng/ml. In the remaining 3 hens, the injection of DOCA resulted in smaller rises of 0.40, 0.49 and 0.87 ng/ml from baseline levels of 1.96, 1.94 and 1.55 ng/ml respectively. In these cases, as judged by examination of oviposition records, ovulation was blocked or delayed by between 24 and 54 h.

c. Oestrone and oestradiol-17β

The timing of oviposition was unaffected in 34 of 37 hens injected with 0.01, 0.1 or 1.0 mg/kg oestrone or oestradiol-17β between 0 and 9 h after ovulation, or 22 to 26 h after the terminal ovulation of a sequence (page 71). Oviposition of the egg in the oviduct at the time of injection was delayed by 2 h and 48 h in 2 of the remaining 3

hens, when injections of 1.0 mg oestrone/kg were given 2.5 h and 9.5 h, respectively, after a predicted ovulation. In the third hen, oviposition was delayed by 5 h following an injection of 0.1 mg oestrone/kg 8.5 h after a predicted ovulation.

d. Androstenedione

Oviposition was unaffected in 18 hens injected with either 0.1, 0.5 or 1.0 mg androstenedione/kg at various times during the ovulatory cycle (page 70).

e. Testosterone

When hens with oviducal eggs were injected with testosterone, oviposition of these eggs was usually delayed by several hours, depending on the dose injected.

(i) Testosterone injections given 0 to 9 h after ovulation

In 3 of 9 hens injected with 0.1 mg testosterone/kg between 0 and 9 h after ovulation, ovipositions were delayed by between 3 and 31 h (mean 12.8 $\pm$ 9.1 (S.E.M.) h). However, in 12 of 15 hens injected with 0.5 mg testosterone/kg and in all of the hens injected with 1.0 (n = 9) and 2.0 (n = 6) mg testosterone/kg, oviposition was delayed by means of 6.6 $\pm$ 1.6 h, 13.9 $\pm$ 5.2 h and 26.7 $\pm$ 8.9 h respectively. These delays (range 2 to 48 h) were unrelated to the period between testosterone injection and the preceding ovulation. Unlike the effects of progesterone at this stage of the cycle, the interval between injection and oviposition of the delayed egg was not constant. However, similar to the effects of progesterone, the delayed oviposition often occurred late in the afternoon and became the last of a sequence since no oviposition occurred on the following day; this presumably resulted from a delay or an inhibition of the subsequent

ovulation. The effects of testosterone injections on oviposition between 9 and 22 h after ovulation were not investigated.

(ii) Testosterone injections given 22 to 26 h after the terminal ovulation of a sequence

When 17 hens were injected with 0.5, 1.0 or 2.0 mg testosterone /kg between 22 and 26 h after the Ct ovulation, i.e. between 4 and 0 h before oviposition of that egg, a steep rise in plasma LH levels similar to that observed during a pre-ovulatory surge invariably occurred (Fig. 17). However, an examination of the oviposition records indicated that the first ovulation of a sequence was prematurely induced in only 3 hens. Of the remaining 14 hens, 3 appeared to be unaffected by the injection, whilst in 11 hens the oviposition of the first egg of a sequence occurred between 9 and 48 h later than expected (mean, 28.9 $\pm$ 4.1 h); this may reflect a delay or an inhibition of ovulation, or could possibly be due to a direct effect on the oviduct. When 0.1 mg testosterone/kg was injected between 22 and 26 h after the C_t ovulation, there was no resulting increase in LH secretion (Fig. 17). Oviposition and probably ovulation were delayed by 24 h in only 1 of 5 hens, and times of oviposition in the other 4 hens were unaffected.

Conclusion

In conclusion, of the steroids used in this study, both progesterone and testosterone consistently effected the timing of oviposition. However, in contrast to the effects of testosterone, the timing of an oviposition delayed by an injection of progesterone appeared to be synchronized to either the progesterone injection itself or the next occurring ovulation.

III. REGULATION OF LH SECRETION IN THE OVARIECTOMIZED HEN

It has been shown that in the laying hen injections of progesterone or testosterone, but not of oestrogen, can stimulate LH secretion (pages 58 to 71). Since a rise in the level of LH in the circulation as a result of an injection of synthetic LH-RH (Etches & Cunningham, 1974) or of ovine LH (Shahabi, Bahr & Nalbandov, 1975) can cause a release of progesterone from the ovary, it is possible that when LH secretion is stimulated after injecting either progesterone or testosterone, other steroids which are also capable of exerting a positive feedback effect on LH may be secreted secondarily. In order to examine directly the positive feedback effects of individual steroids on LH secretion, without the complications caused by an endogenous release of ovarian steroids, it was decided to use ovariectomized hens. Details of ovariectomy are given on pages 27 to 30. All animals used in experiments were between 6 months and 3 years old and were of either the Thornber or Shaver strain.

Plasma LH levels in ovariectomized hens ranged between 30 and 80 ng/ml and were therefore 15 to 40 times greater than LH levels in the laying hen. Due to variations between ovariectomized hens in the baseline concentration of LH in the circulation, changes in plasma LH level after injection of steroids were expressed as a percentage of the pre-injection value. In this way, differences between experimental groups could be compared.

Since plasma LH levels in gonadectomized fowl were not often

depressed by withdrawing frequent blood samples (see page 45), it was possible to carry out a study of the negative feedback and positive feedback effects of gonadal steroids on LH secretion.

Summary of experiments

A. NEGATIVE FEEDBACK EFFECTS OF GONADAL STEROIDS ON LH SECRETION

1. Effects of a single injection of gonadal steroids

Twelve ovariectomized hens were given a single intramuscular injection of either 0.1, 0.5 or 5.0 mg progesterone/kg dissolved in a solution of propylene glycol (page 31). A blood sample was taken by venepuncture immediately before injection at 09:00 h and at intervals of 2, 6, 24, 30, 48, 54 and 72 h thereafter. One control hen was treated similarly, but given an injection of propylene glycol solution only. Oestradiol benzoate, dissolved in arachis oil was injected intramuscularly into 5 other ovariectomized hens at a dose of either 0.1 or 0.5 mg/kg. A blood sample was taken by venepuncture immediately before the injection at 09:00 h and at intervals of 6, 24, 30, 48, 54, 72 and 78 h thereafter. One control hen was sampled similarly, but given an injection of arachis oil only.

It was apparent that both progesterone and oestrogen exerted a negative feedback effect on LH secretion, but it was not possible to compare directly the negative feedback effects of the two steroids since oestradiol was injected as an ester and progesterone as the free steroid. Mean changes in plasma LH levels in relation to the time of

Table 13. Changes in plasma LH concentration (as a percentage of pre-injection values) after the injection of progesterone (P) or oestradiol benzoate (OB) (means ± S.E.M.)

	Dose (mg/kg body wt)	No. of hens	Pre-injection plasma LH (ng/ml)	Time after injection (h) 0	2	6	24	30	48	54	72	78
P	0.1	4	57.4 ± 4.9	100	86.6 ± 4.7	86.0 ± 4.0	85.9 ± 5.3	89.2 ± 3.4	92.3 ± 6.6	92.9 ± 6.9	83.5 ± 3.5	
	0.5	4	59.2 ± 4.3	100	89.7 ± 6.6	81.2 ± 4.8	64.1 ± 14.3	67.6 ± 8.6	82.7 ± 13.8	89.1 ± 8.5	90.4 ± 3.5	
	5.0	4	50.9 ± 8.6	100	108.1 ± 8.2	81.7 ± 11.2	68.8 ± 10.6	68.2 ± 9.0	78.3 ± 18.2	68.0 ± 13.1	85.2 ± 10.2	
Control		1	45.0	100	98.2	96.0	106.0	88.2	105.6	118.2	104.0	
OB	0.1	2	48.4 (35.7, 61.2)	100		93.7 (100.8, 86.6)	93.1 (104.2, 82.0)	87.2 (102.2, 72.2)	83.2 (93.0, 73.5)	86.8 (95.8, 77.9)	75.9 (93.8, 58.0)	87.7 (100.8, 74.7)
	0.5	3	49.2 ± 1.6	100		99.6 ± 0.8	44.0 ± 5.7	43.1 ± 10.8	28.8 ± 7.8	32.1 ± 5.4	32.0 ± 4.5	51.5 ± 4.5
Control		1	35.2	100		88.6	94.5	103.7	102.8	97.2	91.5	

injection of progesterone and oestrogen are shown in Table 13.

2. Effects of multiple injections of gonadal steroids

These experiments were carried out primarily to find a priming schedule which would result in an injection of progesterone exerting a positive feedback effect on LH secretion. Ovariectomized hens were given daily intramuscular injections of progesterone, oestrogen, or combinations of the 2 steroids for periods of up to 2 weeks. The effects of various injection schedules on basal LH secretion were examined in several hens from which blood samples were taken daily before each steroid injection. All gonadal steroids injected during priming were dissolved in 0.3 to 0.5 ml arachis oil.

a. Progesterone priming

Ovariectomized hens were injected on 3 successive days with 0.5 mg progesterone/kg (i.e. 0.5 mg P/kg on days 1, 2, 3). These injections caused a gradual decline in LH levels from 43.7 ± 3.8 ng/ml to 32.0 ± 3.0 ng/ml (Fig. 26).

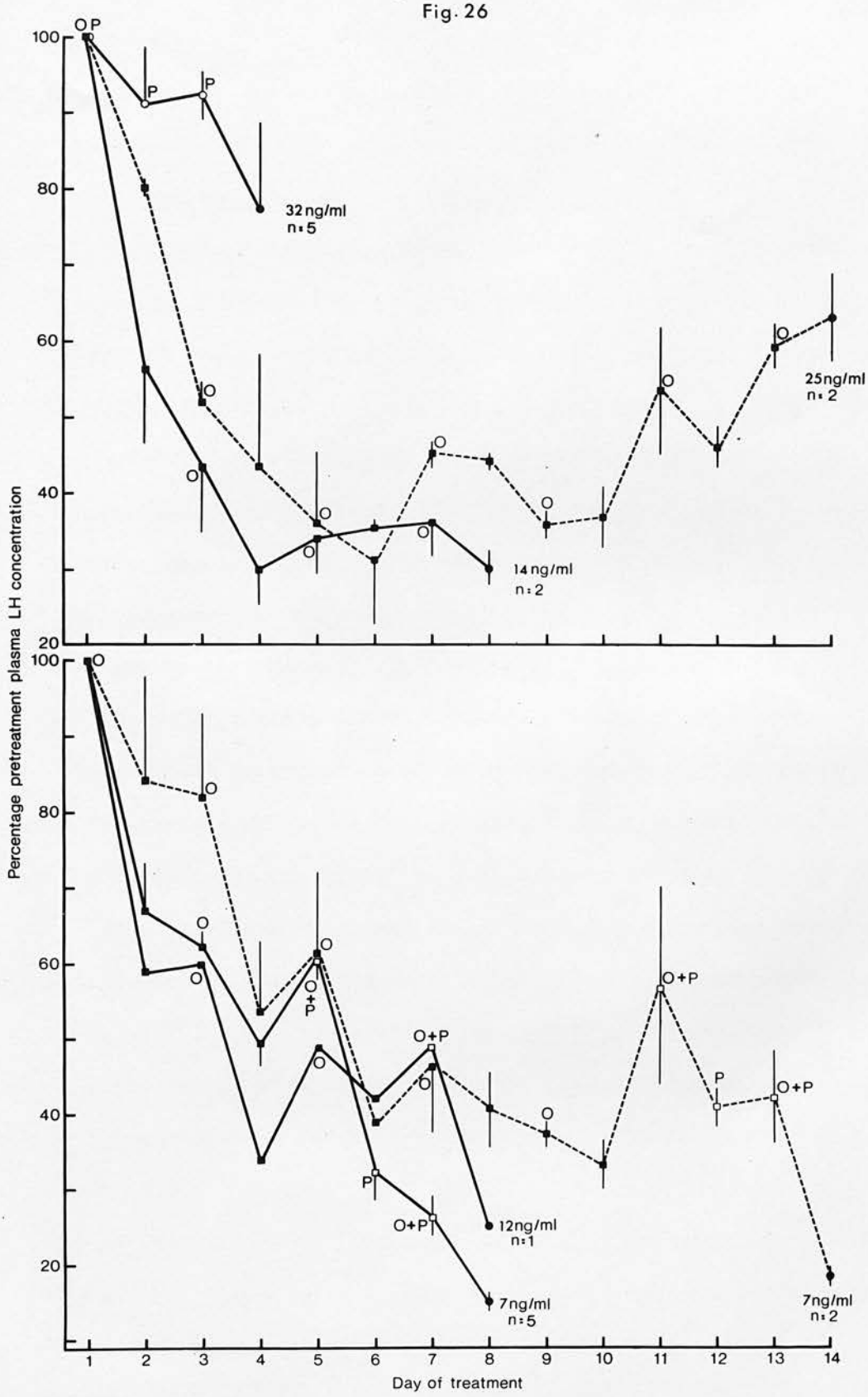
b. Oestrogen priming

A further group of ovariectomized hens were given injections of 0.1 mg oestradiol benzoate/kg on alternate days for either 1 week (i.e. 0.1 mg OB/kg on days 1, 3, 5, 7) or for 2 weeks (0.1 mg OB/kg on days 1, 3, 5, 7, 9, 11, 13). Plasma LH levels in daily samples taken from 2 hens injected for 1 week fell steeply during the first 3 days from a mean of 42.2 ± 0.8 (S.E.M.) ng/ml (n = 2) to 14.1 ± 0.7 ng/ml (n = 2) (Fig. 26). Thereafter, priming injections caused no further depression in LH levels. In 2 hens injected with oestrogen on alternate days for 2 weeks, plasma LH levels fell during the first 5 days of priming from 39.1 ± 5.6 ng/ml to 12.5 ± 5.0 ng/ml. However, during the rest of the

Figure 26

Mean or individual percentage changes in the concentration of plasma LH after intramuscular injections on either successive or alternate days of 0.5 mg progesterone/kg (P), 0.1 mg oestradiol benzoate/kg (O) or both oestradiol benzoate and progesterone (O + P). The days on which injections were given are indicated by the initial letter of each steroid. The mean post-priming LH concentrations are shown following each treatment.

Fig. 26


Percentage pretreatment plasma LH concentration
Day of treatment
32 ng/ml n=5
14 ng/ml n=2
25ng/ml n=2
12ng/ml n=1
7ng/ml n=5
7ng/ml n=2
O
P
O+P

period of priming, LH levels increased gradually, until the mean concentration of LH in the last sample was 24.5 ± 1.5 ng/ml (Fig. 26).

c. Oestrogen + 1 day progesterone priming

An ovariectomized hen was injected with 0.1 mg oestradiol benzoate/kg on alternate days for 1 week and an injection of 0.5 mg progesterone/kg was given together with the last oestrogen injection (0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on day 7). As in the previous experiment oestrogen depressed LH levels during the first 3 days of priming which then remained stable until the injection of progesterone on day 7 caused a further depression to 12.0 ng/ml (Fig.26).

d. Oestrogen + 3 days progesterone priming

Ovariectomized hens were primed with 0.1 mg oestradiol benzoate /kg on alternate days for either 1 week or 2 weeks, and additionally injected with 0.5 mg progesterone/kg on the last 3 days of the priming period (i.e. 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7 or 0.1 mg OB/kg on days 1, 3, 5, 7, 9, 11, 13 + 0.5 mg P/kg on days 11, 12, 13). In 2 hens from which daily blood samples were taken during 13 days of priming, mean LH levels declined from a pre-injection value of 37.0 ± 4.9 (S.E.M.) ng/ml (n = 2) to 12.7 ± 3.5 ng/ml after 5 or 8 days. LH secretion remained at about this level until injection of 0.5 mg progesterone/kg on days 11, 12 and 13 resulted in a further decline to 6.7 ± 0.5 ng/ml (Fig. 26).

A similar fall to 7.1 ± 0.6 ng/ml (n = 5) was observed in the concentration of LH in plasma samples taken from the hens primed with oestrogen and progesterone for 1 week (Fig. 26).

Conclusion

It is evident that injections of 0.5 mg progesterone/kg alone

on 3 successive days exerted a smaller negative feedback effect on LH secretion than did injections of 0.1 mg oestradiol benzoate/kg on alternate days for 1 week (23.3 ± 12.1 (S.E.M.) % (n = 5) versus 69.5 ± 2.5 % (n = 2) reduction) (Fig. 26). However, while injections of 0.1 mg oestradiol benzoate/kg on alternate days no longer depressed LH levels after 4 to 9 days of treatment, subsequent injections of progesterone in combination with oestrogen for 3 days caused the mean LH level to fall by a further 75.5 ± 2.6 % (Fig. 26). It therefore appears that a combination of both steroids exerts a greater depressive effect on LH secretion than either steroid alone.

B. POSITIVE FEEDBACK EFFECTS OF GONADAL STEROIDS ON LH SECRETION

1. Effects of progesterone injections

a. Unprimed hens

Twenty-five ovariectomized hens were given a single injection of either 0.05, 0.1, 0.5, 5.0 or 10.0 mg progesterone/kg, dissolved in 0.3 to 0.5 ml of a propylene glycol solution (page 31). The concentration of plasma LH was measured in blood samples taken by venepuncture at 15 or 30 min intervals for 4.5 to 5.0 h after injection (page 61).

The concentration of LH in the pre-injection blood samples ranged between 32.7 and 65.0 ng/ml (mean, 44.3 ± 1.6 (S.E.M.) ng/ml). Following injection of 0.05, 0.1, 0.5 or 5.0 mg progesterone/kg there was an immediate and consistent fall in LH levels to, respectively, 71.6 ± 5.5 % (n = 3), 68.0 ± 1.8 % (n = 7), 77.3 ± 4.3 % (n = 7) and 81.9 ± 6.7 % (n = 5) after about 1 hour (Fig. 27 a ii - v). In the cases of 0.1 and 0.5 mg progesterone/kg, these falls were significantly different ($P < 0.001$ and < 0.02, respectively) from the LH change observed at 1 h

after the injection of 4 control hens with the carrier (Fig. 27a i), and can therefore be attributed to a negative feedback effect of progesterone on LH secretion. No consistent change was observed following the injection of 10.0 mg progesterone/kg. One hour following the injection of 0.1, 0.5 and 5.0 mg progesterone/kg, mean plasma LH levels rose slightly, though this was only significant ($P < 0.001$) in hens injected with 0.1 mg progesterone/kg. This small rise, which was not observed in the controls (Fig. 27a i) was only transient and failed to reach pre-injection values.

Since a single injection of progesterone in untreated hens did not induce a release of LH, ovariectomized hens were treated with various steroid priming schedules (see pages 108 to 109). Afterwards changes in LH secretion were observed after a single injection of 0.5 mg progesterone/kg dissolved in a solution of propylene glycol (page 31), given on the day following completion of priming treatment. In each case, plasma LH concentration was measured in a blood sample taken by venepuncture immediately before the injection and at 15 and 30 min intervals, as described on page 61 for up to 5 h afterwards.

b. Progesterone-primed hens

Five ovariectomized hens were injected on 3 successive days with 0.5 mg progesterone/kg, as described on page 108. No consistent change in plasma LH concentration was observed after an injection of 0.5 mg progesterone/kg on the day following completion of priming treatment.

c. Oestrogen-primed hens

Eight hens were given injections of 0.1 mg oestradiol benzoate

/kg on alternate days for either 1 or 2 weeks, according to the schedule described on page 108, i.e. 0.1 mg OB/kg on days 1, 3, 5, 7 or days 1, 3, 5, 7, 9, 11, 13. Following an injection of 0.5 mg progesterone/kg on the day after the last priming injection, plasma LH levels fell for 1.0 ± 0.2 h to a mean low of 75.5 ± 6.4 % of the pre-injection level of 16.1 ± 2.8 (S.E.M.) ng/ml. Although the fall was statistically significant ($P < 0.01$), the change in LH level at 1 h after injection was not significantly different from that in the 3 control hens. Between 1 and 2 h after the injection of progesterone, there was a significant rise ($P < 0.001$) in plasma LH concentration which was significantly different ($P < 0.01$) from changes in the concentration of LH in the 3 control hens at that time.

d. Oestrogen + 1 day progesterone-primed hens

Five ovariectomized hens were primed according to the schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on day 7, as described on page 109. In 4 of these hens, 0.5 mg progesterone/kg injected on the day following completion of priming resulted in an increase in plasma LH levels, commencing within 0.25 to 1.0 h after injection. A mean maximal increase of 25.5 ± 12.9 (S.E.M.) % above the pre-injection values of 15.0 ± 2.7 ng/ml was observed 1.7 ± 0.2 h after the injection (Fig. 27d). This increase was statistically significant ($P < 0.05$). In one other hen, plasma LH levels fluctuated erratically and it was not possible to detect a positive feedback response.

e. Oestrogen + 3 days progesterone-primed hens

Eight hens were primed with oestrogen and progesterone for either 1 or 2 weeks according to the following schedules described on

Figure 27

Changes in the concentration of plasma LH in steroid-primed or unprimed ovariectomized hens after a single intramuscular injection of progesterone.

Mean percentage changes in the concentration of plasma LH after a single injection of :-

a different doses of progesterone (ii - vi) or vehicle (i) or 0.5 mg progesterone/kg after :-

b priming with 0.5 mg P/kg on days 1, 2, 3

c priming with 0.1 mg OB/kg on days 1, 3, 5, 7

d priming with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on day 7.

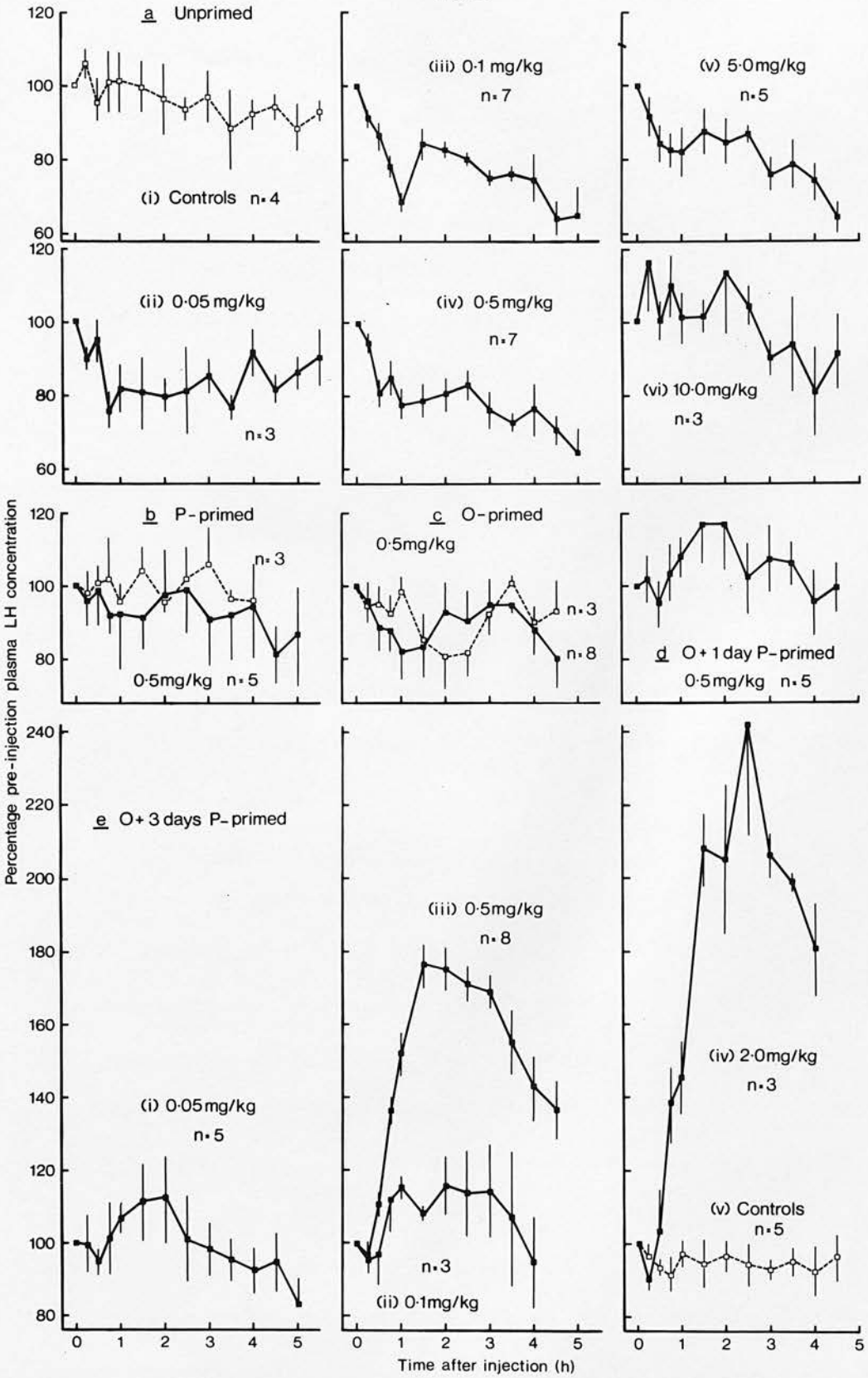
e different doses of progesterone (i - iv) or vehicle (v) in hens primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7.

OB, oestradiol benzoate; P, progesterone.

□----□ , vehicle-injected, steroid-primed controls.

Vertical lines represent ± S.E.M.

Fig. 27


a Unprimed
(i) Controls n•4
(iii) 0·1 mg/kg
n•7
(v) 5·0mg/kg
n•5
(ii) 0·05 mg/kg
n•3
(iv) 0·5mg/kg
n•7
(vi) 10·0mg/kg
n•3
b P-primed
n•3
0·5mg/kg n•5
c O-primed
0·5mg/kg
n•3
n•8
d O+1 day P-primed
0·5mg/kg n•5
e O+3 days P-primed
(i) 0·05mg/kg
n•5
(iii) 0·5mg/kg
n•8
n•3
(ii) 0·1mg/kg
(iv) 2·0mg/kg
n•3
(v) Controls
n•5
Percentage pre-injection plasma LH concentration
Time after injection (h)

page 109. These consisted of either 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7 (n = 6) or 0.1 mg OB/kg on days 1, 3, 5, 7, 9, 11, 13 + 0.5 mg P/kg on days 11, 12, 13 (n = 2). In all cases, injection of 0.5 mg progesterone/kg on the day following the last priming injection caused, within 15 to 30 min, a steep and significant ($P < 0.001$) rise in plasma LH concentration from a mean pre-injection value of 6.9 $\pm$ 0.4 ng/ml to a maximum of 12.7 $\pm$ 0.6 ng/ml (Fig. 27e iii). This maximum increase of between 61 and 110 % (mean 85.5 $\pm$ 5.7 %) was reached 2.1 $\pm$ 0.2 h after injection (Fig. 27e iii). Thereafter, LH levels declined gradually, but were still 36 $\pm$ 8.7 % above pre-injection values 4.5 h after injection.

The time-course of the progesterone-induced LH surge in the oestrogen and progesterone-primed ovariectomized hen was similar to that resulting from an injection of 0.5 mg progesterone/kg in the laying hen (see page 64).

f. Dose-response relationship

Using a priming schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7, as described on page 109, a comparison was made between the LH responses to injections of 0.05, 0.1, 0.5 or 2.0 mg progesterone/kg on the day following the last priming injection. The changes in plasma LH concentrations were measured in blood samples taken by venepuncture at 15 or 30 min intervals (page 61) for a period of 3.5 to 4.5 h after the injection (Fig. 27e i - iv).

In 16 of the 19 hens a slight depression in plasma LH levels was observed during the 15 to 30 min after injection of different doses of progesterone. In all hens injected with 0.05, 0.1, 0.5 or 2.0 mg progesterone/kg, LH levels then began to rise to reach mean

maximal increases of 26.4 ± 7.4 (S.E.M.) % (n = 5), 30.0 ± 5.2 % (n = 3), 85.5 ± 6.0 % (n = 8) and 145.0 ± 28.2 % (n = 3) respectively, above pre-injection values at 2.2 ± 0.4 h, 2.2 ± 0.6 h, 2.1 ± 0.2 h and 2.5 ± 0.0 h after the injection. This rise was significant following injections of 0.1, 0.5 and 2.0 mg progesterone/kg ($P < 0.01$, < 0.001 and < 0.001, respectively). No consistent rise in LH concentration was observed in the 5 control hens.

The largest dose of 2.0 mg progesterone/kg caused the largest LH response, which was significantly greater ($P < 0.01$ than that resulting from injections of 0.5 mg progesterone/kg. This, in turn, was significantly greater ($P < 0.001$) than the response to 0.1 mg progesterone/kg. This can be contrasted with observations in the laying hen, where 0.1 and 0.5 mg progesterone/kg resulted in similar maximal incremental changes in LH levels of 1.55 ± 0.4 ng/ml (n = 7) and 1.73 ± 0.2 ng/ml (n = 7), respectively (page 61).

Injection of 0.05 mg progesterone/kg also exerted a small positive feedback effect on LH secretion. In view of this dose-response relationship, it is apparent that the LH response does not function on an "all or none" principle.

g. Modifications of priming injections

Ovariectomized hens were primed with various combinations and concentrations of oestrogen and progesterone to establish the priming schedule which would result in the LH positive feedback mechanism being maximally responsive to an injection of progesterone.

i) Modifications of progesterone priming

Six ovariectomized hens were primed using the following schedules: 0.1 mg OB/kg on days 1, 3, 5, 7 ± 0.1 mg P/kg on days 5, 6,

7 (n = 3) or 0.1 mg OB/kg on days 1, 3, 5, 7 ± 2.0 mg P/kg on days 5, 6, 7 (n = 3). Both steroids were dissolved in arachis oil. After priming with oestrogen and 0.1 mg progesterone/kg, plasma LH levels fell from a pre-priming level of 49.7 ± 7.2 (S.E.M.) ng/ml to 17.8 ± 8.1 ng/ml. Priming with oestrogen and 2.0 mg progesterone/kg caused a greater decline from 36.3 ± 8.4 ng/ml to 7.3 ± 1.6 ng/ml.

On day 8, the 6 hens were given a single injection of either 0.1, 0.5 or 2.0 mg progesterone/kg dissolved in a propylene glycol solution (page 31) and LH levels were measured in blood samples taken at 15 or 30 min intervals, as described on page 61 , for 4 h afterwards. The resulting changes in plasma LH concentration are shown in Fig. 28a, b. Plasma LH concentrations rose in a series of abrupt fluctuations (Fig. 28a, i, ii, iii) in response to injections of 0.1 (n = 1), 0.5 (n = 1) or 2.0 (n = 1) mg progesterone/kg in hens primed with oestrogen and 0.1 mg progesterone/kg. These observations can be compared with those in hens primed with oestrogen and 0.5 mg progesterone/kg (page 113) in which the mean post-priming LH concentration was 6.9 ± 3.7 ng/ml (n = 8). Here, the LH response to the injection of 0.5 mg progesterone /kg was a smooth, consistent rise in plasma concentration (Fig. 27e iii; Fig. 31c). The maximal increases in plasma LH concentration after a single injection of 0.1, 0.5 or 2.0 mg progesterone/kg in individual hens primed with oestrogen and 0.1 mg progesterone/kg were, 11.0 %, 40.6 % and 12.3 %, respectively, and were reached at 2.75, 2.75 and 2.25 h after the injection. In comparison, increases were less than those in hens primed with the same dose of oestrogen and 0.5 mg progesterone/kg and similarly injected with 0.1, 0.5 or 2.0 mg progesterone/kg. The resulting maximal increases in LH concentration were respectively,

16 to 34 %, 61 to 110 % and 105 to 200 % (Fig. 27e).

After priming injections of 0.1 mg OB/kg on days 1, 3, 5, 7 + 2.0 mg P/kg on days 5, 6, 7, an injection of 0.1, 0.5 or 2.0 mg progesterone/kg on day 8 resulted in a smaller LH response than in similarly injected hens primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7. A single injection of 0.1 mg progesterone /kg failed to increase LH levels above the post-priming value and after a single injection of either 0.5 or 2.0 mg progesterone/kg, LH levels rose to maxima of only 12.7 % and 60.0 % respectively above post-priming values (Fig. 28b).

(ii) Modifications of oestrogen priming

Increased oestrogen

In order to reduce the basal plasma LH concentration, priming doses of oestrogen were increased using injection schedules of 0.1 mg OB/kg on days 1, 2, 3, 4, 5, 6, 7 + 0.5 mg P/kg on days 5, 6, 7 or 0.5 mg OB/kg on days 1, 2, 3, 4, 5, 6, 7 + 0.5 mg P/kg on days 5, 6, 7. These schedules resulted in a steep fall in plasma LH levels from 52.0 $\pm$ 2.4 ng/ml (n = 3) to 3.1 $\pm$ 0.2 ng/ml and from 51.8 $\pm$ 11.9 ng/ml (n = 3) to 1.3 $\pm$ 0.1 ng/ml, respectively. The post-priming LH levels were similar to basal plasma LH levels in the laying hen (page 49).

The effect of priming with a greater amount of oestrogen on the LH response to an injection of 0.5 mg progesterone/kg was variable (Fig. 28c, d). The mean maximal increases in LH concentration after an injection of 0.5 mg progesterone/kg in hens primed with 0.1 or 0.5 mg oestradiol benzoate/kg on successive days for 1 week and 0.5 mg progesterone/kg on days 5, 6 and 7 of that week were 119.7 % (range, 74 to 177 % (n = 3)) and 71.0 % (range, 22 to 156 % (n = 3)) respectively

(Fig. 28c, d). It appears that in some cases, priming with 0.1 or 0.5 mg oestradiol benzoate/kg every day facilitated a larger LH response to progesterone than did priming with 0.1 mg oestradiol benzoate/kg on alternate days. In the latter case, the mean maximal increase in plasma LH concentration following an injection of 0.5 mg progesterone /kg was 85.5 ± 5.7 % (range 61 to 110 % (n = 8)). However, the priming schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7 facilitated a more uniform LH response to an injection of 0.5 mg progesterone/kg in both magnitude and duration than the schedule of 0.1 or 0.5 mg OB/kg on days 1, 2, 3, 4, 5, 6, 7 + 0.5 mg P/kg on days 5, 6, 7.

Reduced oestrogen

An experiment was carried out to determine the effects of priming with less oestrogen by omitting the last 2 oestrogen injections of the priming sequence, i.e. 0.1 mg OB/kg on days 1, 3 + 0.5 mg P/kg on days 5, 6, 7. This reduced LH levels from 36.8 ± 4.6 ng/ml to 20.1 ± 2.1 ng/ml (n = 9). On the day after the last priming injection 6 hens were given a single injection of 0.5 mg progesterone/kg dissolved in a propylene glycol solution (page 31) and 3 other hens were injected with the control solution. The concentration of LH was measured in plasma samples taken before the injection and at 15 or 30 min intervals (page 61) for 4.5 h afterwards. The mean changes in plasma LH level resulting from the injection of progesterone or of the vehicle are shown in Fig. 28e i, ii. A sustained rise in LH level following the injection was observed in only 2 of 6 hens, in which LH levels rose to maxima of 40.6 % and 65.5 % above post-priming values. In the other 3 hens, plasma LH levels fell after injection and remained

Figure 28

Percentage changes in the concentration of plasma LH after a single intramuscular injection of progesterone in ovariectomized hens primed with various combinations of oestrogen and progesterone.

Plasma LH changes after the intramuscular injection of :-

a (i) 0.1, (ii) 0.5 or (iii) 2.0 mg progesterone/kg in hens primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.1 mg P/kg on days 5, 6, 7.

b (i) 0.1, (ii) 0.5 or (iii) 2.0 mg progesterone/kg in hens primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 2.0 mg P/kg on days 5, 6, 7. Vertical lines represent 95 % confidence limits.

Plasma LH changes after the intramuscular injection of 0.5 mg progesterone/kg in :-

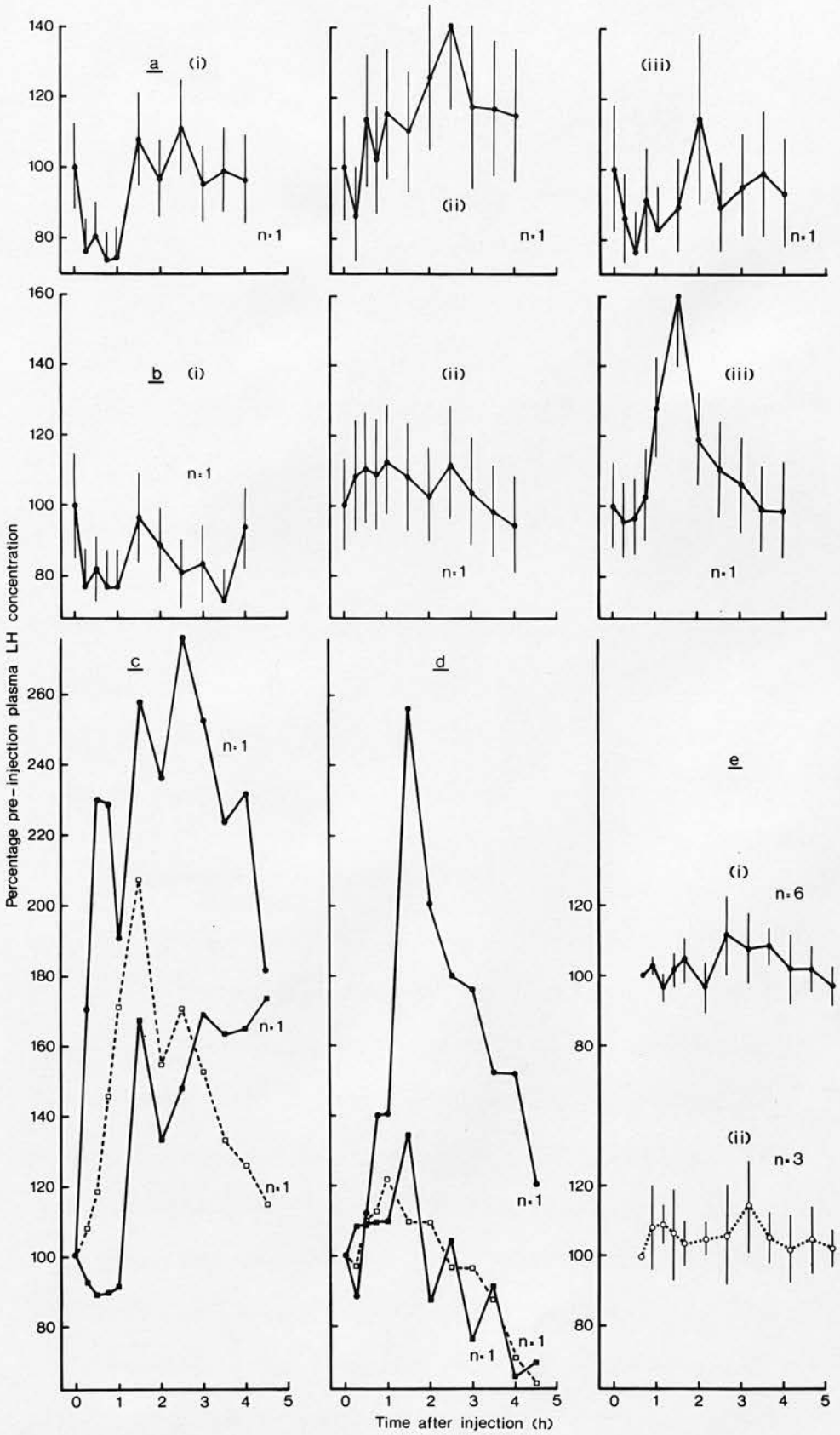
c hens primed with 0.1 mg OB/kg on days 1, 2, 3, 4, 5, 6, 7 + 0.5 mg P/kg on days 5, 6, 7 or

d hens primed with 0.5 mg OB/kg on days 1, 2, 3, 4, 5, 6, 7 + 0.5 mg P/kg on days 5, 6, 7.

e Mean plasma LH changes after the intramuscular injection of (i) 0.5 mg progesterone/kg or (ii) vehicle, in 9 hens primed with 0.1 mg OB/kg on days 1, 3 + 0.5 mg P/kg on days 5, 6, 7. Vertical lines in e represent ± S.E.M.

OB, oestradiol benzoate; P, progesterone.

Fig. 28


a (i)
n=1
(ii)
n=1
(iii)
n=1
b (i)
n=1
(ii)
n=1
(iii)
n=1
c
n=1
n=1
n=1
d
n=1
n=1
n=1
e
(i)
n=6
(ii)
n=3
Percentage pre-injection plasma LH concentration
Time after injection (h)

depressed throughout the sampling period.

It is therefore apparent that sustained priming with oestrogen is necessary to facilitate the LH positive feedback response to progesterone. The priming regime of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7 was chosen as the most suitable for use in further experiments, since the LH response to a single injection of progesterone following this treatment was the most uniform and most closely resembled the natural pre-ovulatory LH surge in the laying hen (cf. Fig. 8; page 49).

Conclusion

It was concluded that priming injections of both oestrogen and progesterone are necessary to facilitate the positive feedback mechanism and that the functioning of this system requires a critical ratio of progesterone to oestrogen in the circulation.

2. Effects of oestrogen injections

Untreated or gonadal steroid-primed ovariectomized hens were given single intramuscular injections of either 0.01, 0.1 or 1.0 mg oestradiol benzoate/kg, dissolved in 0.3 to 0.5 ml of arachis oil. Plasma LH concentrations were measured in blood samples taken by venepuncture immediately before the oestrogen injection and at 15 or 30 min intervals (page 61) for 3.5 to 4.5 h afterwards.

There was no evidence that an injection of oestradiol benzoate caused a rise in LH secretion in either unprimed hens (Fig. 29a), in hens primed with 0.5 mg progesterone/kg on 3 successive days (Fig. 29b), in hens primed with 0.1 mg oestradiol benzoate/kg on 7 successive days (Fig. 29c) or after priming with 0.1 mg OB/kg on days 1, 3, 5, 7 +

Figure 29

Percentage changes in the concentration of plasma LH in gonadal steroid-primed or unprimed ovariectomized hens after a single intramuscular injection of (i) 0.01, (ii) 0.1 or (iii) 1.0 mg oestradiol benzoate/kg in :-

(a) unprimed hens

(b) hens primed with 0.5 mg P/kg on days 1, 2, 3

(c) hens primed with 0.1 mg OB/kg on days 1, 2, 3, 4, 5, 6, 7

(d) hens primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7.

□-----□ , control hens, unprimed or gonadal steroid-primed and injected with vehicle.

(e) hens primed as in (d) and pre-treated 1 h before oestrogen injection with 0.5 mg testosterone/kg.

o·····o , control hens injected with 0.5 mg testosterone /kg alone.

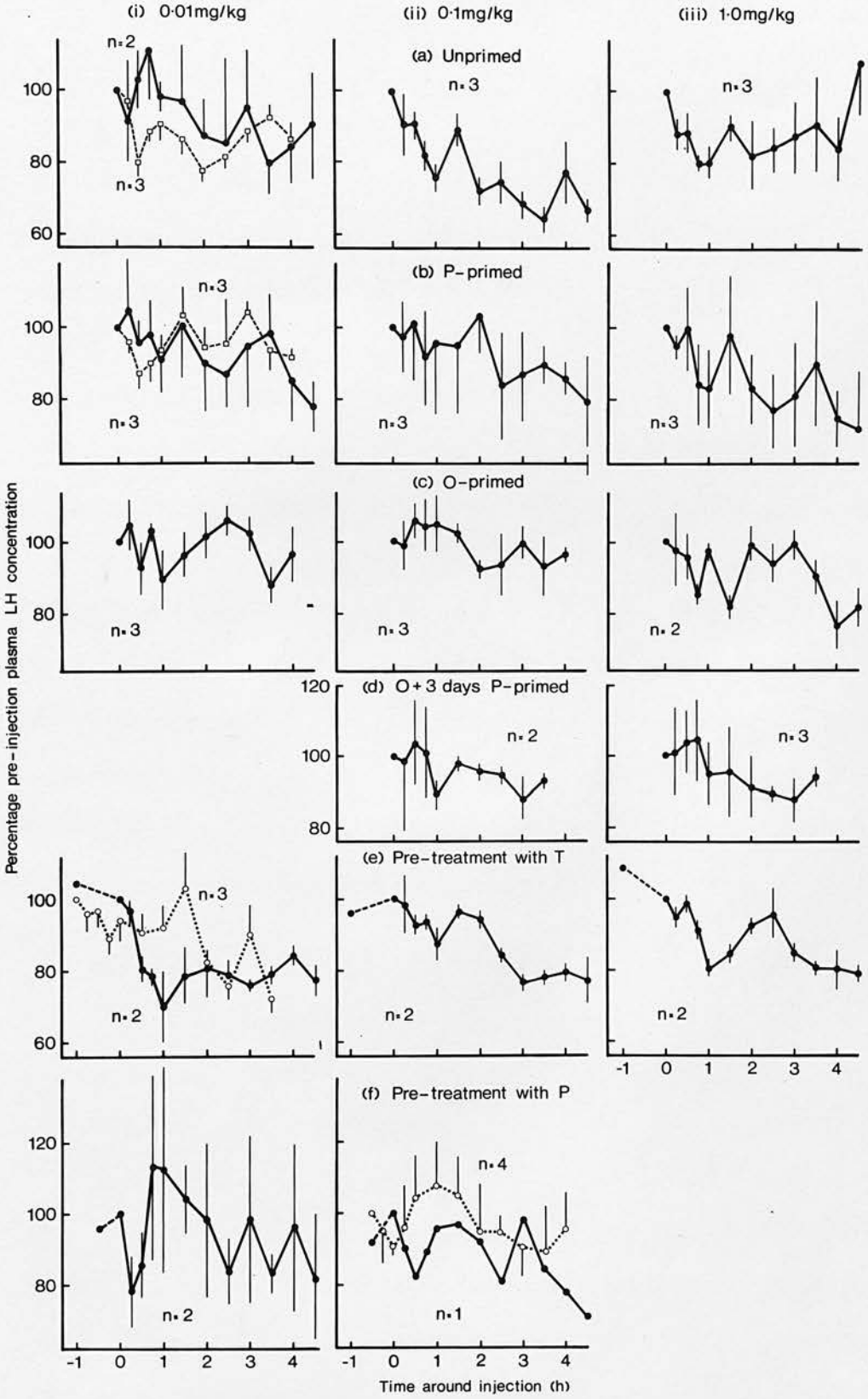
(f) hens primed as in (d) and pre-treated 0.5 h before oestrogen injection with 0.05 mg progesterone/kg.

o·····o , control hens injected with progesterone followed by vehicle instead of oestrogen.

P, progesterone; O, oestradiol benzoate; T, testosterone.

Vertical lines represent ± S.E.M.

Fig. 29


(i) 0·01mg/kg
(ii) 0·1mg/kg
(iii) 1·0mg/kg
(a) Unprimed
(b) P-primed
(c) O-primed
(d) O+3 days P-primed
(e) Pre-treatment with T
(f) Pre-treatment with P
n·2
n·3
n·4
n·1
Percentage pre-injection plasma LH concentration
Time around injection (h)

0.5 mg P/kg on days 5, 6, 7 (Fig. 29d).

It is possible that rising levels of testosterone or progesterone in the circulation, such as occurs between 9 and 2 h before ovulation (page 23) may enable rising levels of plasma oestrogen to exert a positive feedback effect on LH secretion. This possibility was investigated.

Ovariectomized hens were primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7. On the day following completion of priming treatment, 6 hens were injected with 0.5 mg testosterone/kg and 7 with 0.05 mg progesterone/kg. After 1 h, or 30 min, respectively, all hens were injected with 0.01, 0.1 or 1.0 mg oestradiol benzoate/kg. As shown in Fig. 29e and f, oestrogen failed to increase LH levels above those observed in the control hens injected with testosterone or progesterone alone. It was deduced that pre-ovulatory rises of testosterone and progesterone in the circulation do not facilitate any positive feedback effect of oestrogen on LH secretion.

3. Effects of testosterone injections

It has been shown that in the laying hen, an intramuscular injection of testosterone will stimulate LH secretion if given between 22 and 26 h, but not if given between 0 and 9 h after ovulation (page 70). This suggests that testosterone could be acting either directly on the ovary to release progesterone, which in turn stimulates LH secretion, or on the hypothalamo-hypophyseal complex to release LH directly. The possibility that testosterone exerts a positive feedback effect on LH secretion at the central nervous level was investigated using oestrogen and progesterone-primed ovariectomized hens.

Twelve ovariectomized hens were primed using the schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7. This reduced the mean plasma LH concentration from 42.3 ± 3.7 (S.E.M.) ng/ml to 5.7 ± 1.2 ng/ml. On the day following the last priming injection, groups of 3 hens were injected with either 0.1, 0.5, 1.0 or 2.0 mg testosterone/kg dissolved in a propylene glycol solution (page 31). Plasma LH concentration was measured in samples taken immediately before the injection and at 15 or 30 min intervals for 4.5 h thereafter (page 61).

Mean changes in plasma LH concentration after the injection of the 4 dose levels of testosterone are shown in Fig. 30a i - iv. In 10 of the 12 hens plasma LH levels fluctuated erratically and occasionally rose above the pre-injection level: similar fluctuations were not observed in the control hens (Fig. 27e v). However, in 2 hens injected with 1.0 or 2.0 mg testosterone/kg an initial fall in LH concentration was followed by a sustained rise lasting 0.75 to 1.0 h. In these 2 hens LH levels rose to maxima of 19 % and 27 % above the pre-injection values (Fig. 30a v, vi). Although there was evidence in these hens that testosterone exerted a small positive feedback effect on LH secretion, no responses were observed comparable to those occurring consistently in the laying hen following an injection of testosterone at 22 to 26 h after the terminal ovulation of a sequence. This suggests that the injection of testosterone at 22 to 26 h after ovulation causes the release of ovarian progesterone which then stimulates LH secretion.

4. Non-gonadal steroids:- effects of deoxycorticosterone (DOC) injections

It has been shown previously that injections of DOC in the laying hen can stimulate LH secretion when given either between 0 and 9 h after an ovulation or between 22 and 26 h after the terminal ovulation of a sequence (page 68). This suggests that the steroid acts directly on the hypothalamo-hypophysial complex to release LH and was further investigated using ovariectomized hens.

Eight ovariectomized hens were primed with oestrogen and progesterone using the schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7. On the day following the last priming injection, each hen was injected with either 0.5 or 1.0 mg DOCA/kg dissolved in a propylene glycol solution (page 31). Plasma LH levels were measured in samples taken immediately before the injection and at 15 or 30 min intervals for 4.5 h afterwards (page 61). Mean and individual changes in plasma LH concentration after injection of DOCA are shown in Fig. 30b.

Injections of 0.5 and 1.0 mg DOCA/kg caused a rise in LH levels which began within 15 or 30 min of injection and reached maximal levels after 1.6 $\pm$ 0.2 (S.E.M.) h (n = 4) and 2.1 $\pm$ 0.1 h (n = 4), respectively. However, as observed after injection of DOCA in laying hens (page 68), the magnitude of the LH responses varied considerably in different birds. They ranged between an increase of 12.1 and 136.5 % in LH concentration after 0.5 mg DOCA/kg (Fig. 30b i) and an increase of between 75 and 200 % after 1.0 mg DOCA/kg (Fig. 30b ii). Despite the variation in the magnitude of the LH response,

Figure 30

Percentage changes in the concentration of plasma LH after single intramuscular injections of either testosterone or deoxycorticosterone acetate in ovariectomized hens primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7.

a (i - iv) Mean changes in the concentration of plasma LH after injection of 0.1, 0.5, 1.0 or 2.0 mg testosterone/kg,

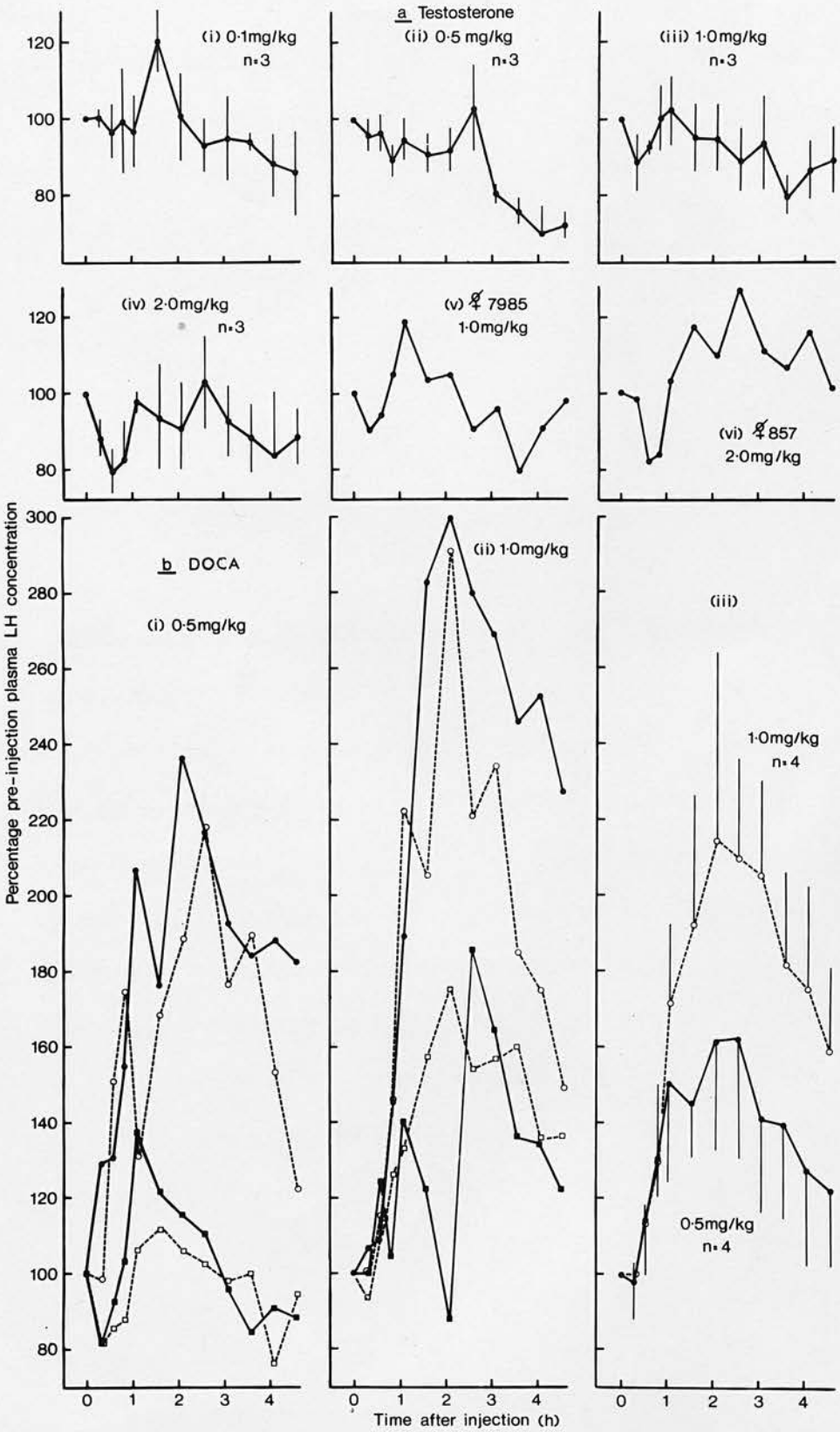
(v - vi) Changes in the concentration of plasma LH in two hens in which injections of either 1.0 or 2.0 mg testosterone/kg caused a rise in LH secretion.

b Changes in the concentration of plasma LH in individual hens after the injection of (i) 0.5 or (ii) 1.0 mg deoxycorticosterone acetate (DOCA)/kg.

(iii) Mean changes in the concentration of plasma LH after injection of 0.5 or 1.0 mg DOCA/kg.

Vertical lines represent ± S.E.M.

Fig. 30


a Testosterone
(i) 0·1mg/kg n·3
(ii) 0·5 mg/kg n·3
(iii) 1·0mg/kg n·3
(iv) 2·0mg/kg n·3
(v) ♀ 7985 1·0mg/kg
(vi) ♀ 857 2·0mg/kg
b DOCA
(i) 0·5mg/kg
(ii) 1·0mg/kg
(iii)
1·0mg/kg n·4
0·5mg/kg n·4
Percentage pre-injection plasma LH concentration
Time after injection (h)

the mean maximal increase in plasma LH concentration following injection of 1.0 mg DOCA/kg (139 ± 33.4 % (n = 4)) was significantly greater ($P < 0.01$) than the increase (76.2 ± 30.2 % (n = 4)) following injection of 0.5 mg DOCA/kg (Fig. 30b iii). The percentage increase in plasma LH concentration in 2 of the 4 hens injected with 1.0 mg DOCA/kg was as great or greater than in any of the 11 hens injected with either 0.5 or 2.0 mg progesterone/kg (293, 200 % versus 61 to 200 % increase) (cf. Figs. 30b i - iii and 27e).

It was concluded that DOC exerts a direct positive feedback effect on LH secretion.

5. Modification of progesterone-induced LH release by gonadal steroids

In studies on modifications of the LH response to a single injection of progesterone by pre-treatment with gonadal steroids in the laying hen (pages 71 to 77), it is possible that the initial steroid pre-treatment stimulated the release of steroids from the ovary. In order to investigate the effects of pre-treatment with oestrogen, testosterone and progesterone on the progesterone-induced LH surge, without the response being modified by endogenously-secreted gonadal steroids, the study was carried out on oestrogen-progesterone primed ovariectomized hens.

Plasma LH levels were measured in blood samples taken before the steroid pre-treatment injections and at 15 or 30 min intervals after the progesterone injection (page 61) for up to 4.5 h. All hens used in this experiment had been primed using the injection schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7.

a. Effects of pre-treatment with oestrogen

On the day following the last priming injection, 9 ovariectomized hens were given a single injection of either 0.01, 0.1 or 0.5 mg oestradiol benzoate/kg dissolved in arachis oil. One hour later, each hen was given a further injection of 0.5 mg progesterone/kg dissolved in a propylene glycol solution (page 31).

There was no evidence that oestrogen pre-treatment further depressed basal plasma LH levels. Changes in plasma LH concentration following the injection of progesterone in each hen are shown in Fig. 31a. It was found that pre-treatment with oestrogen modified considerably the LH response to an injection of 0.5 mg progesterone/kg. The LH responses in 2 control hens pre-treated with an injection of arachis oil followed one hour later with 0.5 mg progesterone/kg consisted of a small decline followed by a gradual rise reaching a peak at 2 or 2.5 h after injection (Fig. 31c). In contrast, the LH response to progesterone in hens pre-treated with 0.1 or 0.5 mg oestradiol benzoate/kg consisted of increased LH levels in the form of abrupt fluctuations (Fig. 31a ii, iii). The least modified LH response followed pre-treatment with the lowest dose (0.01 mg/kg) of oestrogen (Fig. 31a i). The mean maximal percentage increases in LH level in response to an injection of progesterone in hens pre-treated with 0.01, 0.1 and 0.5 mg oestradiol benzoate/kg were respectively, 64.9 (range 42 to 81 %), 50.1 % (range 36 to 78 %) and 84.1 % (range 40 to 108 %) compared with a mean increase of 75 % (83.5, 79.2 %) in the 2 controls pre-treated with arachis oil. In 3 hens pre-treated with 0.1 or 0.5 mg oestradiol benzoate/kg, plasma LH concentration increased by less than 40 % of the level before the progesterone injection. This suggests

that oestrogen pre-treatment modified the positive feedback action of progesterone on LH secretion.

b. Effects of pre-treatment with testosterone

Oestrogen-progesterone primed ovariectomized hens were pre-treated with testosterone to see if this steroid enhanced the LH response to an injection of 0.1 mg progesterone/kg (which normally induces only a small release of LH (page 113)), or modified the normal LH response to injection of 0.5 mg progesterone/kg. Levels of testosterone in the blood have been found to rise at about 9 h before ovulation in the laying hen (Etches, 1974), while the pre-ovulatory rise in plasma progesterone levels begins about 6 h before ovulation (Furr et al., 1973a). Therefore, each of 5 primed hens were given a single injection of 0.5 mg testosterone/kg, followed 2 h later by an injection of either 0.1 or 0.5 mg progesterone/kg. Mean and individual changes in plasma LH concentration following injection of 0.1 (n = 3) or 0.5 (n = 2) mg progesterone/kg are shown in Fig. 31b i, ii. The mean percentage increase in LH levels (13.9 $\pm$ 3.5 % (n = 3)) in hens injected with testosterone and 0.1 mg progesterone/kg was no greater than that observed in 3 hens (30.0 $\pm$ 5.2 %) injected with 0.1 mg progesterone/kg alone (page 113). It was therefore apparent that testosterone pre-treatment did not enhance the LH response to progesterone. Also, no marked difference was observed between the LH response to 0.5 mg progesterone/kg in 2 hens pre-treated with 0.5 mg testosterone/kg and the LH response in 2 control hens pre-treated with an injection of arachis oil (Fig. 31b ii & c).

c. Effects of pre-treatment with progesterone

(i) Pre-treatment 0.75 h earlier.

Eleven oestrogen-progesterone primed ovariectomized hens were given a single injection of either 0.05, 0.1 or 0.5 mg progesterone/kg in a propylene glycol solution (page 31). After 0.75 h, each hen was given a further injection of 0.5 mg progesterone/kg in the same solvent. Mean changes in plasma LH concentration after the injections are shown in Fig. 31d. At 0.75 h after the injection of 0.05, 0.1 or 0.5 mg progesterone/kg, LH levels had fallen by means of 6.1, 5.3 and 6.1 % respectively, of the LH value at the time of the injection. Therefore, the second progesterone injection was given before LH levels had begun to increase consequent to the first injection. The second progesterone injection, in all but 2 of the 11 hens, caused a small rise in LH levels within 0.25 h. However, pre-treatment with progesterone 0.75 h earlier did not modify the LH response to a second injection of 0.5 mg progesterone/kg. The mean maximal increase in LH concentration following the injection of 0.5 mg progesterone/kg in hens pre-treated with 0.05, 0.1 or 0.5 mg progesterone/kg were 85.4 $\pm$ 23.8 % (n = 4) 74.9 $\pm$ 9.0 % (n = 4) and 73.9 $\pm$ 11.8 % (n = 3) respectively, and were similar to increases of 85.5 $\pm$ 5.7 % (n = 8) in oestrogen-progesterone primed ovariectomized hens given a single injection of 0.5 mg progesterone/kg.

(ii) Pre-treatment 4.0 h earlier

Previous experiments in the laying hen showed that 0.5 mg progesterone/kg failed to increase LH secretion when injected on the declining slope of either a natural pre-ovulatory LH surge (page 65) or of an LH surge induced by the injection of progesterone (page 76).

Further findings suggested that this failure to cause a release of LH was not due solely to the high blood concentration of progesterone associated with the LH surge (page 76). It is possible that the pituitary is unable to release LH immediately after the occurrence of an LH surge, or it is possible that rises in the blood concentration of other steroids associated with the pre-ovulatory LH surge in the laying hen, such as oestrogen, may prevent a positive feedback response in LH secretion to injected progesterone.

To test this latter possibility, 6 primed ovariectomized hens were each given an injection of 0.5 mg progesterone/kg dissolved in a propylene glycol solution (page 31). In a blood sample taken at the time of a further injection of 0.5 mg progesterone/kg 4 h later, the plasma LH concentration had risen from 8.3 $\pm$ 2.1 ng/ml (S.E.M.) to 11.5 $\pm$ 2.5 ng/ml (Fig. 31d iv). By comparison with the LH responses in 2 hens injected with 0.5 mg progesterone/kg (Fig. 31c), it was calculated that the second progesterone injection was given on the declining slope of the LH surge initiated by the first injection.

As in the laying hen (pages 65 & 76), the injection of progesterone on the declining LH slope resulted in little or no rise in LH levels. The maximal increase in LH concentration following the second injection of progesterone ranged from 0 to 39.2 % (mean 22.4 $\pm$ 6.1 %) (Fig. 31d iv).

Conclusion

Since there would be no endogenous gonadal steroids released in response to the injection of progesterone or to the rise in LH secretion, it appears that the failure of progesterone to induce LH release on the declining slope of an LH surge was not due to an

Figure 31

Effects of pre-treatment with oestrogen, testosterone or progesterone on the LH response to progesterone in ovariectomized hens primed with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7.

(a) Changes in the concentration of plasma LH in individual hens after a single intramuscular injection of 0.5 mg progesterone/kg preceded 1 h earlier by a single injection of (i) 0.01, (ii) 0.1 or (iii) 0.5 mg oestradiol benzoate /kg.

(b) (i) Mean or (ii) individual changes in the concentration of plasma LH after injection of 0.1 or 0.5 mg progesterone /kg respectively, preceded 2 h earlier, in each case, by a single injection of 0.5 mg testosterone/kg.

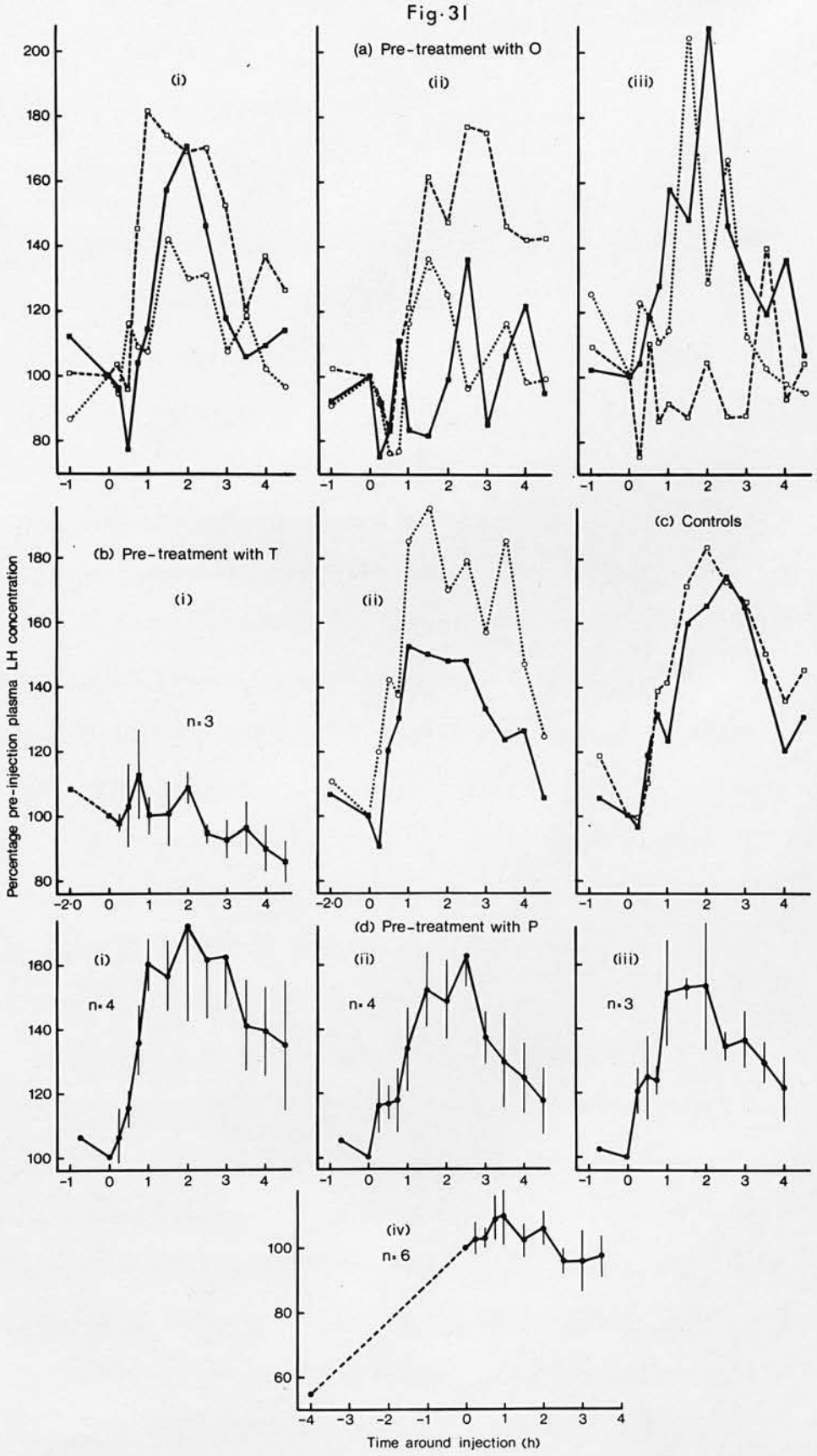
Individual (c) or mean (d) changes in the concentration of plasma LH after a single intramuscular injection of 0.5 mg progesterone /kg :-

(c) preceded 0.75 h earlier by an injection of propylene glycol solution

(d) preceded 0.75 h earlier by a single injection of (i) 0.05, (ii) 0.1 or (iii) 0.5 mg progesterone/kg or (iv) preceded 4 h earlier by a single injection of 0.5 mg progesterone/kg.

O, oestradiol benzoate; T, testosterone; P, progesterone. Vertical lines represent $\pm$ S.E.M.

Fig·31


(a) Pre-treatment with O
(i)
(ii)
(iii)
(b) Pre-treatment with T
(i)
n·3
(ii)
(c) Controls
(d) Pre-treatment with P
(i)
n·4
(ii)
n·4
(iii)
n·3
(iv)
n·6
Percentage pre-injection plasma LH concentration
Time around injection (h)

inhibition of the positive feedback mechanism by gonadal steroids other than progesterone.

6. Effects of injections of gonadal steroids on pituitary responsiveness to LH-RH in the ovariectomized hen.

A study was carried out to determine if changes in the plasma concentration of LH following an injection of progesterone are associated with changes in the responsiveness of the pituitary to LH-RH. This involved comparing the increase in LH secretion after an injection of synthetic LH-RH at various stages of a progesterone-induced LH surge in steroid-primed ovariectomized hens. At the same time the relationship was examined between the negative feedback effects of priming injections of gonadal steroids on LH secretion and changes in sensitivity of the pituitary to LH-RH.

For experiments involving the estimation of pituitary responsiveness to an injection of synthetic LH-RH in the ovariectomized hen, blood samples were taken at 2 min intervals for 16 min by direct venepuncture, as described on page 34.

In order to select the most suitable dose of synthetic LH-RH, 10 ovariectomized hens were injected intravenously with 0.5, 1.0 or 5.0 µg LH-RH/kg. Plasma LH concentrations rose respectively to maxima of 104 ± 18 (S.E.M.) % (n = 3), 262 ± 48 % (n = 3) and 168 ± 36 % (n = 2) of the pre-injection values in respectively, 4.0 ± 0.8 min, 6.5 ± 0.5 min and 7.0 ± 1.0 min. These observations suggested that a dose of 1 µg LH-RH/kg would be suitable for use in subsequent experiments.

Four ovariectomized hens were used in several studies in which a comparison was made between LH responses to synthetic LH-RH injected

intravenously on the day following completion of the priming schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7 and at 0.75 h, 3.25 h or 4.5 h after the injection of 0.5 mg progesterone /kg later on the same day. For 2 of these studies the LH response to injections of synthetic LH-RH was determined at various stages of the same priming schedule.

a. Effects of priming injections of gonadal steroids on pituitary responsiveness to LH-RH

On the day preceding the first priming injection, after an injection of 1 µg LH-RH/kg mean plasma LH values rose within 2 min from a basal level of 38.2 ± 1.7 (S.E.M.) ng/ml (n = 8) to 91.4 ± 4.7 ng/ml after 4.2 ± 0.4 mins (i.e. 242 ± 15 % increase above pre-injection values) (Fig. 32a i). No increase in plasma LH concentration resulted from an injection of 0.4 to 0.6 ml saline in 1 control hen.

On the 4th day of priming with oestradiol benzoate, basal LH levels had fallen to 23.7 ± 2.4 ng/ml (n = 8). Injection of 1 µg LH-RH/kg resulted in an increase in LH concentration to a mean maxima of 43.4 ± 3.8 ng/ml after 4.5 min (i.e. 187 ± 9 % increase above pre-injection levels) (Fig. 32a ii).

A further reduction in LH response to LH-RH was associated with a further depression in the basal level of plasma LH to 6.3 ± 0.7 ng/ml (n = 8) on the day following the last priming injection (day 8). After giving LH-RH, LH levels rose to 21.3 ± 2.1 ng/ml at 4.5 ± 1.0 min (Fig. 32a iii). This was an increase of 356 ± 40 % above pre-injection values. No increase in plasma LH levels was observed in 2 steroid-primed control hens injected with saline. While it appears

that the LH response when expressed as a percentage of the pre-injection value did not consistently alter during priming, the decrease in basal LH secretion following priming injections of gonadal steroids was associated with a reduction in the amount of LH released by an injection of LH-RH.

b. Pituitary responsiveness to LH-RH during a progesterone-induced LH surge.

A preliminary study was carried out to see if one injection of LH-RH modified the response to a second LH-RH injection 5 h later. This was necessary in order to determine if any changes in responsiveness of the pituitary to LH-RH before and during a progesterone-induced LH surge, 5 h later, could be attributed to the LH-RH pre-treatment. Four ovariectomized hens were primed with gonadal steroids according to the schedule 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7. On day 8, each hen was intravenously injected with 1 µg synthetic LH-RH on 2 occasions, separated by a period of 5 h. After the first and second LH-RH injections, LH levels rose respectively by 14.4 ± 1.2 (S.E.M.) ng/ml (n = 4) and 14.6 ± 1.5 ng/ml. In either case, peak LH values were measured at 4 to 6 min after injection. It was therefore concluded that an injection of 1 µg LH-RH did not modify the LH response to a further injection of 1 µg LH-RH given 5 h later.

(i) Response 0.75 h after progesterone injection.

In 4 hens injected with 1 µg LH-RH/kg at 10:00 h on the day following the completion of priming, i.e. day 8, mean plasma LH levels rose from 6.9 ± 0.9 (S.E.M.) ng/ml to maximal values of 21.4 ± 3.6 ng/ml after 3.5 ± 1.0 min. 4.5 h later a further blood sample was

taken from each hen by venepuncture immediately followed by a single intramuscular injection of 0.5 mg progesterone/kg. At the time of the progesterone injection mean plasma LH levels were 6.5 ± 0.9 ng/ml. 0.75 h later LH levels had risen to 9.7 ± 1.1 ng/ml. At this point LH-RH was injected and caused a mean maximal increase of 295 ± 21 % to 28.7 ± 3.8 ng/ml after 10.0 + 2 min (Fig. 32b i). It was therefore evident that the rise in LH levels following an injection of progesterone was associated with an increase in the LH response to synthetic LH-RH (cf. Fig. 32b i and 32a iii).

(ii) Response 3.25 h after progesterone injection.

After allowing 3 weeks for plasma LH levels to rise again after completion of the previous experiment, the same 4 hens were again primed using the schedule of 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7, and on the morning of day 8, each hen was injected with 1 µg LH-RH/kg and plasma LH concentration was measured in blood samples taken for 16 min afterwards. 2.75 h later, each hen was given a single injection of 0.5 mg progesterone/kg. 3.25 h after this, LH levels had risen from 5.8 ± 1.2 ng/ml to only 7.9 ± 1.0 ng/ml (n = 4). This rise of 36.2 % was less than that (60.4 %) observed at the same time after the injection of 8 control hens which had not already been injected with LH-RH (Fig. 27e iii). This indicated that the injection of LH-RH or the resulting rise in LH secretion had modified the LH response to an injection of progesterone 2.75 h later.

Consequently the experiment was repeated, omitting the initial LH-RH test. The 4 ovariectomized hens were primed with oestrogen and

progesterone for 1 week as before, and on the day after the last priming injection each hen was given a single injection of 0.5 mg progesterone/kg dissolved in a propylene glycol solution. 3.25 h later, LH levels had risen from 6.4 ± 1.1 (S.E.M.) ng/ml (n = 4) to 12.0 ± 1.5 ng/ml. It was apparent that the progesterone injection had caused a release of LH, similar in magnitude to that observed in Fig. 27e iii, and it could be assumed that after 3.25 h, LH levels were only just beginning to decline after reaching peak values. At this point, the intravenous injection of 1 µg LH-RH/kg caused LH levels to increase by a maximum of 102 ± 13 % from 12.0 ± 1.5 ng/ml to 24.3 ± 3.5 ng/ml at 5.5 ± 1.7 min after injection (Fig. 32b ii).

(iii) Response 4.5 h after progesterone injection.

In a further experiment, the same group of ovariectomized hens were primed with gonadal steroids for 1 week and then injected with 0.5 mg progesterone/kg. 4.5 h after progesterone injection, LH levels had risen from a mean concentration of 5.7 ± 1.2 ng/ml to 10.5 ± 1.4 ng/ml. It was assumed that an injection of 1 µg LH-RH/kg at this time was given on the middle of the declining slope of the progesterone-induced LH surge. Here, the injection of LH-RH caused LH levels to rise to 21.9 ± 3.4 ng/ml (i.e. 108.3 ± 19.8 % increase in LH levels) (Fig. 32b iii).

At 0.75 h after injection of 0.5 mg progesterone/kg (i.e. on the ascending slope of the induced LH surge) the mean plasma LH level was 9.7 ± 1.1 ng/ml (n = 4) and was similar to the level (10.5 ± 1.4 ng/ml (n = 4) 4.5 h after the injection of 0.5 mg progesterone/kg (i.e. on the descending slope of the induced LH surge). Since the injection

Figure 32

Changes in the concentration of plasma LH following the intravenous injection of 1 µg LH-RH/kg in ovariectomized hens :-

(a) during priming injections of gonadal steroids

(i) before the first priming injection;

(ii) on day 4 after priming with 0.1 mg OB/kg on days 1, 3;

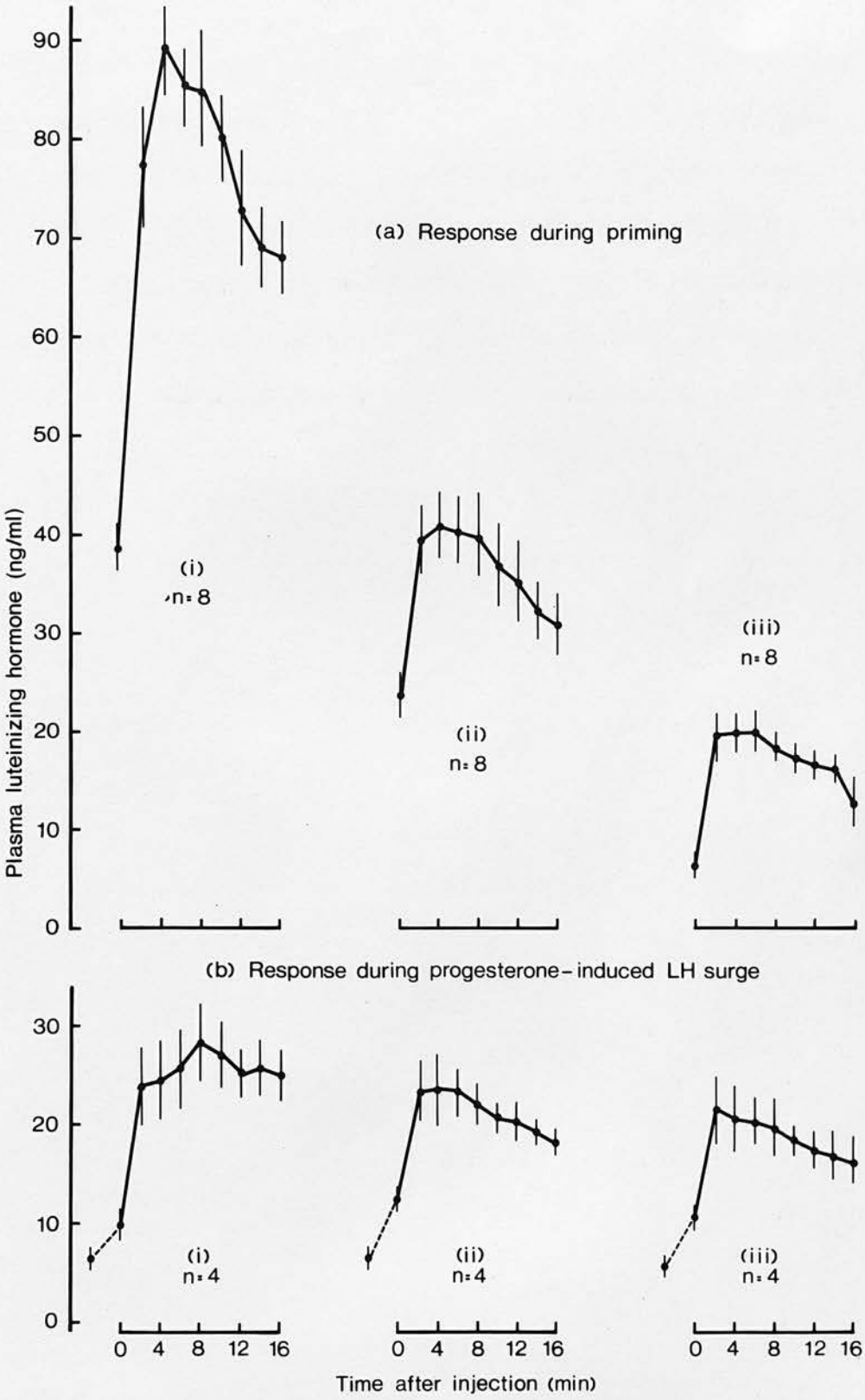
(iii) on day 8 after priming with 0.1 mg OB/kg on days 1, 3, 5, 7 + 0.5 mg P/kg on days 5, 6, 7.

(b) during separate progesterone-induced LH surges in oestrogen-progesterone primed ovariectomized hens

(i) 0.75 h, (ii) 3.25 h, (iii) 4.5 h after an injection of 0.5 mg progesterone/kg.

Dotted lines indicate the change in the concentration of plasma LH between the injection of progesterone and the injection of LH-RH 0.75, 3.25 or 4.5 h later. Vertical lines represent ± S.E.M.

Fig. 32


Plasma luteinizing hormone (ng/ml)
90
80
70
60
50
40
30
20
10
0
(a) Response during priming
(i)
n = 8
(ii)
n = 8
(iii)
n = 8
(b) Response during progesterone-induced LH surge
30
20
10
0
(i)
n = 4
(ii)
n = 4
(iii)
n = 4
0 4 8 12 16
0 4 8 12 16
0 4 8 12 16
Time after injection (min)

of LH-RH on the ascending LH slope caused LH levels to rise to 28.7 ± 3.8 ng/ml compared with a rise to only 21.9 ± 3.4 ng/ml on the descending slope, it is possible that the pituitary was more responsive to LH-RH while levels were rising than when levels were falling. This may partially explain why injections of progesterone are unable to stimulate LH secretion when given on the declining slope of a natural pre-ovulatory (page 65) or progesterone-induced (page 76 and 125) LH peak.

DISCUSSION

I. PATTERNS OF LH SECRETION IN THE DOMESTIC FOWL

The development of a radioimmunoassay for the measurement of LH in the fowl (Follett et al., 1972) makes it possible to detect rapid changes in the level of LH in small volumes of plasma sampled at frequent intervals from individual fowl. It was demonstrated with this technique that LH is released in pulsatile (episodic) discharges. This neuroendocrine control mechanism appears to be common to more than one vertebrate class since a similar pattern of LH secretion has been extensively demonstrated in many mammals, e.g. castrated and ovariectomized rats (Gay & Sheth, 1972; Blake, 1975), guinea pigs (Donovan, ter Haar & Rosenberg, 1975), ovariectomized ewes (Butler, Malven, Willett & Bolt, 1972; Reeves, O'Donnell & Denorscia, 1972), ovariectomized monkeys (Dierschke, Bhattacharya, Atkinson & Knobil, 1970), bulls (Katangole, Naftolin & Short, 1971), male rabbits (Rowe, Hopkinson, Shenton & Glover, 1975; Moor & Younglai, 1975) and humans (Midgley & Jaffe, 1971; Boyar, Perlow, Hellman, Kapen & Weitzman, 1972; Yen, Tsai, Naftolin, Vandenberg & Ajabor, 1972; Santen & Bardin, 1973).

The most striking examples of episodic discharges in the fowl were seen in cockerels where intermittent bursts of LH secretion occurred every 2 to 6 h (Figs. 1, 2; pages 44 to 45). A depression in the mean plasma LH levels noticeable in the cockerels is possibly due to handling of the birds associated with the frequent withdrawal of blood samples and is not likely to be due to haemorrhage since the LH level in such cases fell below that measured at the beginning of the experiment before 5 ml of blood, which is less than 2 % of the total blood volume, was withdrawn. This steep depression in LH levels has

not been reported in most studies of episodic secretion in mammals, though acute stress in the male rat as a result of handling (Krulich, Hefco, Illner & Read, 1974), or in the ovariectomized rat stressed as a result of immobilization (Blake, 1975), have resulted in a drop in LH concentration in the circulation (Krulich et al., 1974; Blake, 1975) and an inhibition of pulsatile discharges (Blake, 1975). In view of the depressive effect of handling on LH secretion in experimental cockerels, it must be considered that the observed magnitude and frequency of episodic secretion may not be the same as in unhandled birds. The apparent absence of an episodic release of LH in two cockerels (page 44) may mean that such episodes were of too low an amplitude to be detectable. This would account for a lack of fluctuations over a 2 h period in saline-injected intact cockerels reported by Furr, Onuora, Bonney & Cunningham (1973) since in that study pre-injection LH levels were 2 to 4 ng/ml, which is low compared with the mean values of 7 to 16 ng/ml observed in the present study in infrequently handled cockerels (page 44). It is possible that low mean plasma LH levels can be maintained by a mechanism not involving the pulsatile release of the hormone.

The apparent lack of a pulsatile release of LH which was observed in the hen may be connected with the way in which ovarian secretions modify the release of LH. It was noticeable that even in one hen which was sampled at frequent intervals during the descending slope of the pre-ovulatory peak of LH, and therefore at a time when LH levels were high compared with other stages of the cycle, no episodic pattern of LH secretion could be detected (Fig. 5). The absence of episodic discharges on the descending slope of a pre-ovulatory LH surge

was also noted in the rhesus monkey (Weick, Dierschke, Karsch, Butler, Hotchkiss & Knobil, 1973) and is in contrast to observations in women in whom episodic release has been demonstrated throughout the ovulatory cycle and with increased amplitude during the pre-ovulatory LH peak (Midgley & Jaffe, 1971; Yen et al., 1972a). The possibility that episodic discharges of LH could be detected when pre-ovulatory levels of LH were rising in the hen was not investigated.

An episodic pattern of LH secretion was also not observed in the immature hen. This may have been due to the rapid fall in plasma LH levels during the initial sampling period (Fig. 6). However, it is possible that the higher level of LH in the immature hen, in contrast to the mature hen (Fig. 23) is associated with a pulsatile pattern of LH secretion, although this could not be demonstrated. Pulsatile discharges of LH have been shown to occur in the immature sow (Foxcroft, Pomerantz & Nalbandov, 1975) and in the immature lamb in which LH levels are higher than those of the adult (Foster, Jaffe & Niswender, 1975). Episodic secretion may thus be associated with the mean level of LH in the blood or may be a common feature of the immature animal, irrespective of the level of LH.

As in mammals, removal of the ovaries or testes from the fowl results in increased levels of plasma LH which are maintained by intermittent secretory episodes. The interval between these episodes (20 to 45 min) is very similar to that demonstrated in gonadectomized rats (Gay & Sheth, 1972; Blake, 1975) but is less than in ovariectomized sheep (45 to 74 min, Butler et al., 1972; Reeves et al., 1972), ovariectomized rhesus monkeys (approximately 75 min, Dierschke et al., 1970) or in a castrated man (approximately 50 min, Santen &

Bardin, 1973). The possibility that LH levels are higher in gonadectomized than in intact adult fowl because of differences in the biological half-life of the hormone can be discounted since it was found that the half-life of ^{125}I-labelled fowl LH was between 18 and 22 min in both intact and gonadectomized birds (page 48).

On comparing the episodic release pattern of LH in intact cockerels and gonadectomized fowl, it appears that the interval between the secretory peaks is related to the mean plasma LH level. For example in cockerel no. 7128 (Fig. 1) in which the mean plasma LH level was 6.9 ng/ml, there were 1 to 2 secretory episodes every 3 h whereas in the castrated cockerel no. 9144 (Fig. 4) in which the mean plasma LH level was 49.4 ng/ml, there were 4 to 5 pulsatile discharges over the same period. This suggests that in the fowl, the increased plasma LH levels observed after gonadectomy are caused chiefly by an increase in the frequency of the pulsatile release of the hormone from the pituitary.

The steep rise and more gradual decline in plasma LH levels characteristic of a secretory episode are identical with the changes in plasma LH levels in cockerels which follow a single intravenous injection of synthetic LH-releasing hormone (Furr, Onuora, Bonney & Cunningham, 1973). The half-life of endogenous (episodic) LH (18 to 25 min) was similar to the biological half-life of ^{125}I-labelled LH as estimated by Scanes & Follett (1973) (mean 21.9 min) and as estimated in this study (18 to 22 min) (page 48). It was noticeable that the disappearance in the bloodstream of either ^{125}I-labelled LH or endogenous (episodic) LH could be described by a two-phase exponential curve (Fig. 7). This may reflect a distribution of LH in 2 components.

The similarity in the rate of decay of both ^{125}I-labelled and episodic LH suggests that LH secretion ceases entirely during the declining phase of a secretory episode. The pulsatile pattern of LH release may be a consequence of intermittent and abrupt discharges of LH-RH, as is suggested by the observations of Osland, Gallo & Williams (1975). They found that the superfusion of rat anterior pituitary fragments with a constant level of a medium containing synthetic LH-RH results in a constant rate of LH release while brief stimulation with LH-RH causes the pulsatile discharge of LH. Alternatively, episodic release may be a reflection of the mechanism by which the pituitary releases LH in response to a constant rate of secretion of LH-RH. This is supported by the observation that episodic secretion of LH continued in the rhesus monkey after the pituitary stalk had been sectioned (Knobil, 1974).

II. THE REGULATION OF BASAL LH SECRETION

There is considerable experimental evidence to suggest that LH levels are lower in intact than in gonadectomized mammals as a result of a suppression of LH synthesis and secretion by gonadal steroids (Davidson, 1969).

LH levels are several-fold higher in adult ovariectomized hens (30 to 80 ng/ml) than in intact hens (1 to 3 ng/ml) which suggests that ovarian steroids exert a negative feedback effect on LH secretion in the hen. The 15- to 30-fold increase in LH levels after ovariectomy in the hen is similar to the 20-fold rise in rats (Gay & Sheth, 1972) and 10-fold rise in the rhesus monkey (Atkinson et al., 1970).

In the ovariectomized hen, single or multiple injections of either oestrogen or progesterone caused a reduction in plasma LH levels (page 107); however, it is not known if the doses of steroids used in this study were physiological. The negative feedback effects of oestrogen were not as immediate as those of progesterone which may be partially due to the fact that oestradiol was injected as an ester and progesterone as the free steroid (page 107; Table 13). Of these two steroids, oestrogen appeared to exert the stronger depressive effect on LH levels (page 110). This agrees with the observations that oestrogen is more effective in reducing LH levels than progesterone in the immature sow (Foxcroft et al., 1975), ovariectomized rhesus monkey (Yamaji, Dierschke, Bhattacharya & Knobil, 1972) and in the agonadal woman (Wallach, DeCherney, Russ, Duckett, Garcia & Root, 1973). In the rhesus monkey (Yamaji et al., 1972) and ewe (Diekman & Malven, 1973), the decline in LH levels following injection of oestrogen was associated with a reduction in magnitude and frequency of episodic discharges. In the guinea pig, the injection of progesterone also inhibits pulsatile discharges of LH (Donovan, ter Haar & Rosenberg, 1975) though this effect is not observed in the rhesus monkey (Yamaji et al., 1972).

Sensitivity to the negative feedback effect of oestrogen in the ovariectomized hen was reduced after injections of the steroid on alternate days for 4 to 9 days. Despite the continued administration of oestrogen, LH levels began to rise gradually although they were still low compared with levels before oestrogen treatment was started (page 108). A similar change in sensitivity of the negative feedback mechanism has been observed in the rhesus monkey (Karsch, Weick,

Hotchkiss, Dierschke & Knobil, 1973). In the ovariectomized hen when there appeared to be a decrease in sensitivity of the negative feedback mechanism, and where the injection of the same dose of oestrogen could be considered sub-threshold, progesterone injections appeared to synergize with oestrogen to exert a stronger suppressive effect than either steroid alone (page 110). This synergism between oestrogen and progesterone has been previously demonstrated in the ovariectomized rat (McCann, 1962) and ovariectomized rhesus monkey (Karsch et al., 1973c) and in the agonadal woman (Wallach et al., 1973) where progesterone only exerts a negative feedback effect on LH secretion after prior treatment with oestrogen.

Although injections of gonadal steroids in the ovariectomized hen reduced both basal LH secretion and responsiveness of the pituitary to synthetic LH-RH (page 128), the percentage increase in plasma LH concentration after the injection of LH-RH did not decrease during priming. It is therefore likely that the reduction in basal LH secretion was not entirely due to decreased pituitary sensitivity to LH-RH, but was the consequence of reduced LH-RH secretion together with a reduction in pituitary responsiveness to LH-RH.

III. LH SECRETION DURING SEXUAL MATURATION

A decrease in sensitivity to the negative feedback effects of gonadal steroids has been postulated to occur at puberty in mammals (Hohlweg, 1936; Donovan & van der Werff ten Bosch, 1959). In the immature animal, the negative feedback receptors at the hypothalamus

are considered to be very sensitive to gonadal steroids and a low level of steroid secretion by the immature ovary can suppress LH secretion. However, shortly before puberty there is an increase in the threshold of the negative feedback receptors in the hypothalamus to gonadal steroids. This results in an increase in the secretion of LH-RH, leading to a greater release of LH. Ovarian growth is thereby stimulated and gonadal steroids are released in greater quantities until a new equilibrium between the secretion of gonadal steroids and LH is reached.

There is experimental evidence suggesting in mammals that a decrease in the negative feedback effects of gonadal steroids on LH secretion occurs after sexual maturation (in the human - Grumbach, Roth, Kaplan & Kelch, 1974; in the rat - Smith & Davidson, 1968; Eldridge et al., 1974).

A rise in basal LH secretion around the time of sexual maturation has been noted in the guinea pig (Donovan, ter Haar, Lockhart, MacKinnon, Mattock & Peddie, 1975), in the heifer (Swanson, Hafs & Morrow, 1972), in the human (Franchimont & Gaspard, 1973; Faiman & Winter, 1974) and in the rhesus monkey (Dierschke, Weiss & Knobil, 1974). Some authors observed a small rise in LH levels in the circulation shortly before the first oestrous in the rat (Meijs-Roelofs, Uilenbroek, Osman & Welschen, 1973), but others reported no change (Ojeda & Ramirez, 1972).

It was evident that dose-levels of oestrogen which were insufficient to depress LH secretion in the laying hen resulted in a fall in plasma LH levels when injected into pullets about 3 weeks

before their first oviposition (page 86). However, it was not possible to study the sensitivity to the negative feedback effects of gonadal steroids on LH secretion in pullets at earlier stages of development since the withdrawal of blood samples in such birds at 6 h intervals resulted in a depression of LH and any negative feedback effects of gonadal steroids were presumably masked.

When the concentration of LH was measured in plasma samples taken twice-weekly from individual hens during the period from 14 weeks of age to the onset of lay, a 2- to 4-fold rise in LH levels was found to occur at about 8 to 12 weeks before lay, in association with an increase in the rate of comb growth (page 83). Since comb growth is dependent on gonadal steroid secretion (Parkes & Marshall, 1960) and plasma oestrogens have been shown to rise steeply about 6 weeks before the onset of lay (Peterson & Webster, 1974; Senior, 1974), it is likely that levels of gonadal steroids in the circulation were rising at the same time.

The pre-pubertal increase in LH secretion was not due to an enhanced sensitivity of the pituitary to LH-RH since there were similar incremental changes in LH concentration after an injection of 10 μg LH-RH/kg before and during the period of increasing basal LH secretion. As the maximal incremental change in LH concentration after an injection of LH-RH remained stable while basal LH levels were rising then the percentage increase in LH secretion after LH-RH injection must have been falling. Consequently, the pituitary was becoming increasingly insensitive to releasing factor at this time. It is therefore likely that any decrease in the sensitivity of the

negative feedback mechanism regulating LH release by gonadal steroids occurred solely at the hypothalamic negative feedback receptor sites and that rising plasma LH concentration was the result of increased secretion of LH-RH.

The pre-pubertal rise in LH secretion in the hen may stimulate the increased rise in the blood level of oestrogen which follows and probably accounts for the development of the oviduct at that time. However, Peterson & Webster (1974) and Senior (1974) observed that oestrogen levels in the blood were rising throughout development in pullets, apart from the 3 weeks preceding onset of lay, and it is therefore possible that rising blood levels of oestrogen over a sustained period caused a reduction in the negative feedback effects of steroids and stimulated the pre-pubertal increase in LH secretion. This may be comparable to the situation in the adult ovariectomized hen, where sensitivity to injected oestrogen was reduced after prior treatment with the steroid (page 108). It is known that subcutaneous implants of oestradiol in immature guinea pigs over 10 days of age stimulate the pre-pubertal increase in LH secretion (Donovan, ter Haar & Peddie, unpublished observations, cited in Donovan, ter Haar, Lockhart, MacKinnon, Mattock & Peddie, 1975).

Bonney, Cunningham & Furr (1974) reported a decrease in pituitary sensitivity to LH-RH during development. However, their findings differ from those described here, since they observed a decrease in magnitude of LH response when LH-RH was given to hens of 11 to 21 weeks of age in which pre-pubertal LH levels were rising. Although the pituitary may be becoming increasingly insensitive when basal LH levels are rising, it is likely that the apparent reduction

in response to LH-RH in the study of Bonney et al., (1974) was due partly to not adjusting the amount of LH-RH injected to take into account changes in body weight with increasing age.

Bonney et al., (1974) found that highest LH levels occurred in pullets of 21 weeks of age, at about 2 weeks before the onset of lay. In contrast, in the present study, the rising levels of LH reached a peak at about 4 weeks before lay and thereafter declined steadily to minimum values at the time of the first oviposition. In this study, LH levels were related to the stage of ovarian development or to the onset of lay, whereas Bonney et al., (1974) related LH levels to the age of the pullet. Since the hens in the present study started laying at times varying between 22 and 28 weeks of age, it is apparent than hens of the same age may vary considerably in their degree of sexual development. This may explain the discrepancy between the changes in the basal level of LH in the study of Bonney et al., (1974) and the present study.

Falling LH levels during the 4 weeks before the onset of lay were associated with a reduction in pituitary sensitivity to LH-RH. This was not due to the negative feedback action of rising blood levels of oestrogen since these are falling during the same period (Peterson & Webster, 1974; Senior, 1974). The fall in LH levels was associated with a marked increase in the rate of yolk deposition and since rapidly-growing, yolky ovarian follicles secrete progesterone (Furr, 1969) it is possible that the blood level of progesterone was increasing during the 3 to 4 weeks before lay. As shown on page 110 progesterone alone is not a strong inhibitor of LH secretion, but it is possible that an increase in the blood level of progesterone

synergized with oestrogen in the circulation to exert an increased negative feedback effect on LH secretion.

IV. LH SECRETION DURING THE OVULATORY CYCLE

The measurement of luteinizing hormone in the circulation at intervals of 1.5 h throughout the ovulatory cycle of the hen revealed a single major surge of secretion, the peak values of which occurred 4 to 5 h before the time of a predicted ovulation (Fig. 8; pages 49 to 51). The pre-ovulatory LH surge, which was of 5 to 8 h duration, consisted of a gradual rise lasting 2 to 3 h, followed by a similar decline. Since the rate of decrease was much less than that observed following an episodic discharge of LH (cf. Figs 8 & 2) or the intravenous injection of ^{125}I-labelled LH (page 48) it is considered that LH secretion was continuing on the declining phase of the surge.

The only other consistent change in LH level observed during the ovulatory cycle was a small increase at the onset of darkness, which in several cases formed a small peak. This rise in plasma LH concentration was observed in all but 1 of 23 hens studied and occurred regardless of whether or not an ovulation was expected that night. This is discussed further on page 191 in relation to the regulation of ovulation sequences.

Around the time of oviposition there was an increase in plasma LH concentration in 5 of 15 hens in the present study. A similar small increase was reported by Cunningham & Furr (1972) at 20 to 23 h before ovulation in 5 of 24 hens in which LH levels were also measured by radioimmunoassay. A peak of OAAD activity was also observed at 20

to 23 h before the third ovulation of a sequence (Nelson et al., 1965; Bullock & Nalbandov, 1967). However, the OAAD bioassay has been shown to measure an "arginine vasotocin-like substance" (Frankel et al., 1965) and it is known that neurohypophysial hormones are released at the time of oviposition (Sturkie & Lin, 1966).

The occurrence of a single major pre-ovulatory LH peak is in agreement with observations of Furr et al. (1973a) who, using the same radioimmunoassay, observed a peak of LH secretion at 7 to 4 h before ovulation, and confirms the observations of Fraps and co-workers who predicted that there is an interval of about 6 h between the release of ovulation-inducing hormone and ovulation on the basis of experiments involving hypophysectomy, lesioning the hypothalamus and injecting LH or progesterone at various times before a predicted ovulation (see pages 11 to 14).

No major rise in LH secretion was observed in hens which were not expected to have an ovulation during or shortly after the sampling period. This is inconsistent with the hypothesis of Bastian & Zarrow (1955) who proposed that there was a sustained rise in LH levels for a period of about 8 h each night, including the night when no ovulation was expected.

There was no evidence to suggest that the amount of LH released before ovulation was related to the position of a particular ovulation in a sequence, though there was some suggestion that pre-ovulatory LH peaks are of greater magnitude in hens which lay long sequences than in those with short sequences, e.g. hens 4154, 4133 (Fig. 11b; page 55).

A single pre-ovulatory peak of LH has also been detected in all mammalian species in which the level of the hormone in the blood has

been determined throughout the ovulatory cycle (review; Brown-Grant 1971).

V. THE REGULATION OF CYCLIC LH SECRETION

Generally, in all mammalian species so far investigated, the LH peak at oestrous is preceded or accompanied by a surge in the circulating concentration of oestrogens, while progesterone levels only start to rise after the LH surge has been initiated (page 4). In contrast, in the hen concentrations of testosterone, oestrogen and progesterone increase in the blood prior to or in association with the pre-ovulatory LH surge. It is considered from the present study that either or all of these steroids are involved in the induction of LH release. The reasons are discussed below.

A. EVIDENCE FROM EFFECTS OF INJECTIONS OF ANTISERA TO GONADAL STEROIDS ON THE PRE-OVULATORY LH PEAK.

In the rat, passive neutralization of oestrogen by the injection on dioestrous 2 of an antiserum raised against that steroid prevented the pro-oestrous surge of LH and subsequent ovulation (Ferin et al., 1969; Neill et al., 1971). In contrast, the injection of an antiserum raised against progesterone failed to influence LH release (Ferin et al., 1969). Passive immunization therefore seemed a suitable method of investigating the control of cyclic LH release in the hen. However, in this study the injection of laying hens with antisera to oestrogen, progesterone or testosterone at about 12 to 16 h before an expected ovulation did not completely inhibit the pre-

ovulatory LH surge (pages 54 to 58). Consequently, a definite conclusion could not be drawn as to the steroid or steroids directly responsible for initiating the pre-ovulatory LH surge.

However, observations on the effects of antiserum injections were not completely negative. The injection of antiserum to progesterone delayed the LH peak in 50 % of cases and, after increasing the titre of antiserum by doubling the volume injected, there was both a delay and a reduction in magnitude of the LH peak (page 57). Since an injection of antiserum to progesterone failed to inhibit completely the rise in plasma LH levels resulting from an intramuscular injection of 0.5 mg progesterone/kg, it is possible that the titre of antiserum injected was sufficient to neutralize only partially the biological effects of the pre-ovulatory surge in progesterone. In view of the observation of Furr & Smith (1975) that an injection of antiserum to progesterone blocked ovulation in 7 of 13 hens, it seems likely that a sufficient titre of antiserum to progesterone would have inhibited a pre-ovulatory discharge of LH.

A single intravenous injection of an antiserum raised against oestradiol-17β at 12 to 16 h before a predicted ovulation did not appear to either modify or delay the pre-ovulatory LH surge associated with that ovulation (page 55). This is consistent with the observations of Furr & Smith (1975) who failed to affect ovulation in any of 10 hens injected with antiserum to oestradiol. The comparatively large LH surges in 2 oestradiol-antiserum injected hens (Nos. 4154, 4133; Fig. 11) may have been related to the length of the egg sequence for each hen, rather than to the effects of antiserum. These 2 hens had sequences consisting of 8 to 10 eggs and 12 to 19

eggs, respectively, whereas most of the hens in this experiment had sequences of about 4 to 5 eggs. The absence of an effect on the LH surge following a single injection of antiserum to oestradiol suggests that the pre-ovulatory rise of oestrogen in the circulation is not directly involved in initiating the LH surge which it accompanies (Senior & Cunningham, 1974; Lague et al., 1975).

When an intravenous injection of antiserum to oestradiol was given on 2 successive days, the pre-ovulatory LH surge expected to occur after the second injection was delayed and reduced in magnitude. It was interesting to observe that an LH rise of only 100 % was sufficient to induce an ovulation (page 56) suggesting that the normal pre-ovulatory LH increase of about 200 % is in excess of that required to induce ovulation. In view of the observation (page 117) that reduction of oestrogen priming in oestrogen-progesterone primed ovariectomized hens reduced the positive feedback effect of progesterone on LH release, it is likely that the inhibition of oestradiol uptake into the hypothalamo-hypophysial complex for 2 days interrupted a facilitative effect that oestrogen may exert on the induction of LH release by progesterone.

The effects of an injection of antiserum to testosterone on the occurrence of a pre-ovulatory LH surge and ovulation were variable (page 54). The injection of antiserum did not inhibit the occurrence of the LH peak and in only 1 of 4 hens did the pre-ovulatory LH peak appear to be smaller than those seen in the control hens. However, in 3 of 4 hens an injection of antiserum to testosterone interrupted the laying pattern. This may have resulted from an interference with oviduct motility, though since in 2 hens, no eggs were laid on 2 or 3

consecutive days following injection, it is likely that testosterone antiserum delayed or prevented ovulation from occurring. These findings suggest that testosterone may be normally involved in regulating ovarian development rather than be directly involved in the positive feedback mechanism regulating cyclic LH secretion. It is possible that the antiserum to testosterone may have modified a pre-ovulatory surge of FSH secretion, an effect which has been shown to occur in rats (Gay & Tomacari, 1974). A disruption of FSH secretion may then have affected the integrity of the maturing ova.

One of the main drawbacks to the use of passive immunization methods to study the role of gonadal steroids in the regulation of cyclic LH secretion is that it is difficult to use a negative observation to draw any firm conclusions. For example, it is not known if the antiserum did actually neutralize the circulating steroids. Also, neutralization of gonadal steroids in the circulation may cause a compensatory overstimulation of the secretion of that steroid from the ovary, or a disruption of the normal pattern of secretion of other steroids. For these reasons, observations from the study of the effects of injections of gonadal steroids on the pattern of LH secretion was perhaps of more value, and will now be discussed.

B. THE INDUCTION OF LH RELEASE

1. Effects of progesterone and oestrogen

Ever since Rothchild & Fraps (1949b) showed that progesterone-induced ovulation is dependent upon the presence of the pituitary gland, it has been accepted that this steroid exerts a positive feedback effect on the secretion of LH. By measuring directly the changes in

circulating levels of LH after injections of progesterone during the ovulatory cycle in the present study it has been possible to confirm this view. The injection of 0.5 mg progesterone/kg at all stages of the ovulatory cycle except during the 4 h period preceding ovulation caused a release of LH, the characteristics of which were similar when injections were given in the period between 4 h after one ovulation and 12 h before the next.

The characteristics of an LH surge induced by an intramuscular injection of 0.5 mg progesterone/kg differed from a natural pre-ovulatory LH surge (cf. Figs. 13 & 8) since LH levels rose more quickly (1 to 1.5 h versus 2.0 to 2.5 h) and showed a smaller mean maximal incremental change (1.58 $\pm$ 0.10 (S.E.M.) ng/ml (n = 37) versus 2.35 $\pm$ 0.17 ng/ml (n = 11)) than during a spontaneous surge. These differences suggest that after an intramuscular injection of 0.5 mg progesterone/kg, the concentration of the steroid in the blood rises more rapidly than before a naturally-occurring ovulation. In hens given an intraperitoneal injection of progesterone, which might be expected to result in a more gradual release of the steroid into the circulation, LH levels rose more slowly over a period greater than 4.5 h (page 59). This is a more extended rise in LH secretion than occurs during a natural pre-ovulatory surge. It is possible that when the circulating levels of progesterone become too high, such as after an intramuscular injection of 0.5 mg progesterone/kg, the hypothalamo-hypophyseal complex may become increasingly refractory and unable to release LH in response to progesterone. This is suggested by the observation that 0.5 mg progesterone/hen, which is approximately half the dose used in this study, occasionally increased plasma levels of

the steroid to about twice the normal pre-ovulatory concentration (Etches & Cunningham, 1975). Additionally, an intramuscular injection of 0.5 mg progesterone/kg caused a smaller release of LH when preceded by an injection of 0.5 mg progesterone/kg 50 min earlier than when preceded by an injection of 0.1 or 0.05 mg progesterone/kg (page 74).

In both mammals and the hen a rise of oestrogen in the blood precedes the pre-ovulatory LH surge. In contrast to observations in the hen, in mammals there is other evidence for the positive feedback effect of oestrogen on LH release. For example, the injection of antibodies to oestrogen blocks the pre-ovulatory LH surge, while injections of antibodies to progesterone are ineffective (Ferin et al., 1969). Also, single or multiple injections of oestrogen will induce an LH discharge in the anoestrous ewe (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969; Scaramuzzi, Caldwell & Moor, 1970) and in the rat at dioestrous (Brown-Grant, 1969; Schwartz, 1969) or during the follicular phase of the menstrual cycle in the rhesus monkey (Dierschke, Yamaji, Karsch, Weick, Weiss & Knobil, 1973) and human (Yen & Tsai, 1972). These rises are similar in magnitude and duration to the natural pre-ovulatory peak of LH for each species. In contrast, in the hen injections of between 0.01 and 1.0 mg/kg of either oestradiol-17β or oestrone at various stages of the ovulatory cycle failed to stimulate LH secretion. Since progesterone induced LH release when injected at any stage of the ovulatory cycle apart from the 4 h preceding an ovulation, it is probable that in the hen, progesterone and not oestrogen is responsible for initiating the pre-ovulatory LH surge.

Progesterone can also exert a positive feedback effect on LH

secretion in the rat, but as far as is known, only at a time when oestrogen levels in the blood are rising. Thus an injection of progesterone induces LH release in the rat when given at pre-oestrous, whereas when progesterone is injected a day earlier at dioestrous there is a decrease in plasma LH concentration (Brown-Grant & Naftolin, 1972) and the next expected ovulation is delayed by 1 or 2 days (Brown-Grant, 1969). This is in contrast to the ovulation-inducing effects of an injection of oestrogen at dioestrous (Brown-Grant, 1969).

Many studies in mammals have shown that priming injections of steroids are necessary before a single injection can stimulate the release of LH. In the ovariectomized rat, single or multiple priming injections of oestrogen followed by a single injection of oestrogen (Caligaris, Astrada & Taleisnik, 1971a), progesterone (Caligaris, Astrada & Taleisnik, 1971b; Kalra, Kalra, Krulich, Fawcett & McCann, 1972) or testosterone (Brown-Grant, 1974) result in increased LH secretion. A single injection of oestradiol benzoate following priming injections of progesterone evokes a release of LH in the ovariectomized ewe (Pelletier & Signoret, 1969; Scaramuzzi, Tillson, Thorneycroft & Caldwell, 1971).

However, a few studies have indicated that priming injections are unnecessary in order to obtain a positive feedback response to oestrogen. A single subcutaneous injection of oestrogen induced a release of LH in the untreated ovariectomized rat on the afternoon of the first (Legan & Karsch, 1974) or second day (Burnet & MacKinnon, 1975) following the injection. Also, a single injection of oestradiol benzoate has been found to induce an LH surge in unprimed ovariectomized ewes (Pelletier & Signoret, unpublished observations,

cited in Pelletier, 1971).

In contrast, no LH response was observed within 4 to 5 h following an intramuscular injection of progesterone in the ovariectomized hen. Here the injection of progesterone was followed by an immediate fall in plasma LH levels which, after the injection of 0.1 and 0.5 mg progesterone/kg, was significantly different from LH changes in the control hens (page 110). The negative feedback effects of progesterone lasted for about 1 h after the injection of 0.1 to 5.0 mg/kg. After 1 h, LH levels rose slightly, which may reflect a small positive feedback response, but no increase above the pre-injection concentration was observed. A dose of 10.0 mg progesterone/kg was not followed by a fall in plasma LH levels and it is possible that progesterone exerted a positive feedback effect of sufficient strength to cancel out the negative effects of the steroid. If rising progesterone levels in the blood had been maintained for longer duration, by a more gradual release of the steroid into the blood, then a single injection of progesterone may have caused a release of LH. It has been shown in the rhesus monkey that both strength and duration of stimulus are important in the induction of LH release (Karsch, Weick, Butler, Dierschke, Krey, Weiss, Hotchkiss, Yamaji & Knobil, 1973), and it is noticeable that where a single subcutaneous injection of oestrogen induced an LH surge in the ovariectomized rat (Legan & Karsch, 1974), the injection resulted in elevated blood levels of oestrogen for several hours.

In contrast to observations in the mammal, in the ovariectomized hen, multiple injections of either progesterone or oestrogen alone did not enable a single intramuscular injection of

progesterone to stimulate LH and the resulting pattern of LH secretion was similar to that observed following an injection of progesterone in unprimed ovariectomized hens (Fig. 27a, b, c). It was apparent that priming injections of both steroids were required and that the magnitude of the LH response depended on both the dose of steroids injected during the priming schedule and the duration of priming treatment with each steroid. Thus, injections of oestrogen for 1 week and a single injection of progesterone on the last day of the week facilitated an LH rise of 25 % in response to a further injection of 0.5 mg progesterone/kg on the following day. However, where priming injections of progesterone were extended to the last 3 days, a further injection of 0.5 mg progesterone/kg on day 8 caused an 85.5 % increase in LH concentration (Fig. 27d, e; page 113).

Since only negative feedback effects of progesterone were observed in unprimed ovariectomized hens, it is apparent that the negative and positive feedback effects of a single steroid injection were independent of each other and that the positive feedback response was superimposed on the negative feedback effect. The immediate negative feedback effect of progesterone on LH secretion, together with the more delayed positive feedback response, accounted for the biphasic appearance of the LH response to an injection of progesterone, a feature which has been observed in several mammals (Scaramuzzi et al., 1971; Karsch et al., 1973b; Legan & Karsch, 1974)

The priming schedule of intramuscular injections of 0.1 mg oestradiol benzoate/kg on alternate days for 1 week together with injections of 0.5 mg progesterone/kg on days 5, 6, and 7 of that week was found to be optimal for a single injection of 0.5 mg progesterone

/kg to exert a positive feedback effect on LH release.

In the ovariectomized hen there was a clear dose-response relationship between progesterone injected and LH released. After the injection of each dose, plasma LH concentration started to rise within 15 to 45 min and reached a maximum after 1 to 3 h. This confirms the suggestion made by Rothchild & Fraps (1949b) that progesterone induces an immediate release of LH. The immediate rise in LH secretion following the injection of progesterone is similar to the close temporal relationship between the pre-ovulatory rises of progesterone and LH in the blood (Furr et al., 1973a) and provides evidence to suggest that progesterone secretion normally initiates the pre-ovulatory LH surge in the hen. Van Tienhoven (1954) suggested that there was a minimum period of 1.5 h during which the pituitary must remain intact following the injection of progesterone for an ovulation to be induced. It would appear from the present study that the interval of 1.5 h is that required for LH levels to rise to around peak values after an injection of progesterone (Fig. 27e).

An increase or reduction in the dose-level of either steroid during priming injections generally modified the LH response to a single injection of progesterone on the day following the last priming injection. When the dose-level of progesterone used in priming was reduced to 0.1 mg/kg, basal plasma LH levels fell to 17 ng/ml and the rise in LH levels after a single injection of 0.5 mg progesterone/kg was composed of abrupt fluctuations (Fig. 28a). Where the dose-level of progesterone used in priming was increased to 2.0 mg/kg, basal LH levels fell to a mean of 7.3 ng/ml and the positive feedback response to progesterone was abolished. However, in hens primed with an

intermediate dose level of 0.5 mg progesterone/kg, LH levels fell to a similar 6.85 ng/ml, and yet an injection of 0.5 mg progesterone/kg on the day after the last priming injection induced a 60 to 110 % increase in plasma LH levels. It seems therefore that the lack of a positive feedback response after priming with 2.0 mg progesterone/kg was not due to insufficient stores of LH in the pituitary, and may reflect a hypothalamic refractoriness to the positive feedback effects of progesterone as a result of excessive levels of progesterone in the blood. Also, since the LH response to an injection of 0.5 mg progesterone/kg in hens primed with oestrogen and 0.1 mg progesterone /kg was reduced compared with that in hens primed with 0.5 mg progesterone/kg (40.6 % versus 85.5 % increase), it is apparent that the normal sensitivity of the positive feedback system in laying hens is dependent on a critical concentration of progesterone in the blood.

Increased dose-levels of oestrogen used in priming, in some cases increased the response to progesterone, while in other cases the positive feedback response was reduced (page 116 ; Fig. 28c). The withdrawal of oestrogen priming from days 5 to 7 of the oestrogen-progesterone priming schedule in ovariectomized hens abolished the positive feedback response to an injection of progesterone on day 8 (page 117). In view of this effect, it appears that the continued secretion of oestrogen is necessary to facilitate the positive feedback effects of progesterone and that an appropriate balance of concentrations of both steroids in the blood is necessary for the expression of a mature positive feedback response. This conclusion is supported by the observation that neutralization of oestrogen by the injection of antiserum to oestradiol-17β on 2 successive days both

delayed and considerably reduced the magnitude of the natural pre-ovulatory LH surge (page 56). It is possible, therefore, that the pre-ovulatory rise of oestrogen in the blood primes the positive feedback mechanism to enable it to respond to a surge of circulating progesterone with secretion of sufficient LH-RH to release quantities of LH from the pituitary sufficient to cause ovulation. Also, since in the ovariectomized hen the positive feedback response to an injection of progesterone was tested 24 h after the last priming injection of oestrogen (page 113), it is apparent that in the laying hen the oestrogen surge preceding the pre-ovulatory LH surge is not required for that LH surge to be initiated. This is supported by the observation that a single injection of antiserum to oestradiol-17β at 12 to 16 h before an ovulation, failed to modify the LH surge associated with that ovulation. The pre-ovulatory oestrogen surge may contribute to sustaining a critical blood concentration required for maintaining sensitivity of the positive feedback system. It is probable that oestrogen plays no direct role in initiating the pre-ovulatory LH surge, since intramuscular injections of 0.01 to 1.0 mg oestradiol benzoate/kg failed to stimulate LH secretion in either unprimed ovariectomized hens or in ovariectomized hens treated with various priming schedules consisting of multiple injections of oestrogen, progesterone or both steroids (page 118). This lack of effect is unlikely to have resulted from an inadequacy of priming in ovariectomized hens since injections of oestradiol-17β or oestrone at doses of 0.01, 0.1 or 1.0 mg/kg failed to stimulate LH release in the laying hen. Since circulating levels of testosterone and progesterone, as well as oestrogen are also high at 9 to 2 h before ovulation, it was

thought that oestrogen might exert a positive feedback effect on LH release in the presence of rising levels of other gonadal steroids. In principle this sort of mechanism may occur in the rat where progesterone will only induce LH release when oestrogen levels are rising (see page 151). However, pre-treatment with either steroid did not enable oestrogen to exert a positive feedback response on LH secretion (page 119).

It thus seems unlikely that oestrogen is directly responsible for initiating the pre-ovulatory LH surge, but it is possible that it may modify the positive feedback effects of rising progesterone levels. This is suggested by the observation that an intramuscular injection of primed ovariectomized hens with 0.01 to 1.0 mg oestradiol benzoate /kg reduced the LH response to a single intramuscular injection of 0.5 mg progesterone/kg 1 h later. Perhaps oestrogen competes with progesterone for positive feedback receptor sites. In contrast, injections of similar doses of oestrogen and progesterone in the laying hen did not result in a reduced LH response. An explanation may be provided by the observation that primed ovariectomized hens were less sensitive to the positive feedback effects of progesterone than laying hens. In the laying hen 0.5 mg progesterone/kg caused a maximal response whereas in the ovariectomized hen, the same dose caused a submaximal response (cf. pages 62 & 114). It is possible that progesterone could compete more adequately for positive feedback receptor sites with a submaximal than a maximal dose of progesterone.

2. Effects of deoxycorticosterone

The effects of injections of 0.1 to 1.0 mg deoxycorticosterone

acetate/kg (DOCA) on stimulating LH release when given at most stages of the ovulatory cycle, or when given to oestrogen-progesterone primed ovariectomized hens were similar to those observed after the injection of progesterone.

The LH response to DOCA was expected since Fraps (1955b) found that ovulation was prematurely induced in 15 of 17 hens (87 %) injected with 1.0 mg of the steroid at 14 h before the expected time of the first ovulation of a sequence. Similarly-timed injections of 1.0 mg progesterone/hen induced ovulation in 18 of 19 hens (95 %) (Fraps, 1955b). After giving either DOCA or progesterone, any resulting ovulation was induced within 8 h of injection. Fraps (1955b) suggested that DOCA acted as a weak progestin since deoxycorticosterone is structurally similar to progesterone and also possesses progesterone-like activity when administered systemically to mammals (Gros, Benoit, Kehl & Paris, 1942; Hooker & Forbes, 1949). However, other studies have suggested that the progesterone-like activities of DOCA are the result of its conversion in the adrenal and kidney to progesterone (Lazo-Wasem & Zarrow, 1955). Supporting evidence for this comes from the observation that injections of DOCA caused an elevation in serum progesterone levels in gonadectomized monkeys (Zarrow, Hisaw & Bryans, 1950) and gonadectomized rats (Lazo-Wasem & Zarrow, 1955). However, the conversion rate of less than 1 % (Lazo-Wasem & Zarrow, 1955) suggests that the increase in blood progesterone levels resulting from the injection of between 0.1 and 1.0 mg DOCA/kg in the present study would be insufficient to induce LH release and it is more probable that DOC was itself exerting a positive feedback effect on LH secretion.

After progesterone is injected into the oestrogen-progesterone primed ovariectomized hen or at any stage of the ovulatory cycle apart from the 4 h period preceding ovulation, plasma LH levels begin to rise within 45 min. Similarly, whenever injections of DOCA stimulated LH secretion, plasma LH levels started to rise after the same time interval (pages 121 & 68). As in the case of progesterone, injections of DOCA could equally stimulate LH secretion irrespective of whether they were given 0 to 9 h or 22 to 26 h after ovulation. However, DOCA was not as uniformly effective as progesterone in causing the release of LH in the laying hen since the magnitude of the LH response was variable and not related either to the dose of DOCA or to the stage of the cycle at which the steroid was injected (page 68). This variation could be due to differences among individual hens in the ability of the positive feedback receptor sites in the hypothalamus to distinguish between progesterone and deoxycorticosterone.

In the oestrogen-progesterone primed ovariectomized hen, again the LH responses to the injection of DOCA were variable in magnitude, and it was found that LH levels rose higher in some hens injected with 1.0 mg DOCA/kg (Fig. 30b) than in hens injected with either 0.5, 1.0 or 2.0 mg progesterone/kg (Fig. 27e). This may reflect a more gradual metabolism of the esterified DOC which would extend the period during which it could exert an influence.

The pattern of deoxycorticosterone secretion throughout the ovulatory cycle has not yet been determined, and it remains to be shown whether this steroid has any physiological role in triggering the pre-ovulatory LH surge. However, a possible role for adrenal steroids in the control of LH release and ovulation cannot be

disregarded. There is some evidence of a role for the adrenal gland in ovulation in rats (Feder, Brown-Grant & Corker, 1971; Lawton, 1972; Mann & Barraclough, 1973). Van Tienhoven (1961a) induced ovulation in the hen by injecting large doses of ACTH, an effect assumed to be mediated through the adrenal gland. Further evidence for a role of the adrenal was supplied by Soliman & Huston (1974) who found that dexamethasone, which is known to block ACTH release from the pituitary, could prevent ovulation when injected at 14 h before the first expected ovulation of a sequence. In these cases, the blocking effect was overcome by the injection of either ACTH or a combination of corticosterone and progesterone, suggesting that adrenal steroids may be involved in initiating the pre-ovulatory LH surge.

3. Effects of testosterone

Fraps (1955b) observed ovulation in 13 of 32 (41 %) hens injected with 1 mg testosterone at about 14 h before the first expected ovulation of a sequence. The interval between injection and ovulation was always greater than 9 h. In contrast, in the same study, the interval between injection of 1 mg progesterone/hen and ovulation was always less than 8 h, suggesting that progesterone and testosterone were not acting in the same way. This observation led him to suggest that testosterone may have to be converted to an "active substance" before ovulation could be induced (Fraps, 1955b).

Following the intramuscular injection of 0.5, 1.0 or 2.0 mg testosterone/kg between 22 and 26 h after the final ovulation of a sequence, plasma LH levels rose to a peak similar to the naturally-occurring pre-ovulatory LH surge (cf. pages 70 & 49). No comparable

LH surge resulted from testosterone injections between 0 and 9 h after ovulation or in ovariectomized hens primed with oestrogen and progesterone for 1 week. It therefore appears that the ability of testosterone to stimulate LH release depends on the functional state of the ovary. The concentration of progesterone in the largest ovarian follicle increases 6- to 7-fold about 19 h after the previous ovulation (Shahabi, Norton & Nalbandov, 1975) and it is possible that testosterone injected after this time causes a release of ovarian progesterone, which then exerts a positive feedback effect on LH secretion. The characteristics of the LH surge following testosterone injections are indicative of a natural release of progesterone since LH levels rose gradually as during a natural pre-ovulatory surge (cf. Figs. 17 iii and 8a, b). This contrasts with the steep rise in LH levels resulting from a single intramuscular injection of progesterone (cf. Figs. 17 iii and 13). As no increase in LH secretion was observed following injections of testosterone between 0 and 8 h after ovulation, the ovarian follicle, at this stage of the ovulatory cycle, may not have been able to secrete sufficient progesterone to stimulate the release of LH.

The mechanism by which testosterone could stimulate secretion of progesterone is unclear. It could either act directly on the follicle to enable it to release progesterone or exert a weak positive feedback effect on the hypothalamo-hypophyseal complex and cause a minor increase in plasma LH levels. There was some evidence to suggest that testosterone could exert a small positive feedback effect on LH release in the oestrogen-progesterone primed ovariectomized hen (page 120). Increased plasma LH levels resulting from the injection of synthetic LH-RH (Etches & Cunningham, 1975) or ovine LH (Shahabi, Bahr & Nalbandov,

1975) are known to stimulate progesterone secretion in the hen, and the ability of a small increase in LH levels in the circulation to trigger an initial release of progesterone in the hen may depend on the amount of this steroid stored in the follicle next due to ovulate. The small increase in plasma LH levels after the injection of testosterone at 8 to 9 h after a first ovulation of a sequence probably reflects the amount of progesterone released from the follicle. Shahabi, Bahr & Nalbandov (1975) induced steroidogenesis in follicles by injecting ovine LH at 12 h after the occurrence of an ovulation which resulted in rises of testosterone and progesterone in both the follicles and the circulation at 30 min after injection. It must, therefore, be deduced that any small initial rise in LH levels that the testosterone injection may have brought about was insufficient to stimulate steroidogenesis within 8 h of the previous ovulation.

If testosterone caused a small release of LH in the laying hen, it may not have been observed because it was too small or was masked by the larger rise in LH levels presumably caused by the positive feedback action of progesterone released from the ovary. Another possibility is that testosterone differentially stimulated FSH secretion, which then caused the release of progesterone from the maturing ovarian follicle. A role for androgens in maintaining the pre-ovulatory rise in FSH secretion in the rat has been suggested by Gay & Tomacari (1974). If testosterone does cause a release of gonadotrophins via a central nervous positive feedback mechanism, it may as Fraps (1955b) suggested have to be first converted into an "active substance". It has been shown that testosterone can be metabolized to androstenedione in both the brain (Nakamura & Tanabe,

1974) and ovary (Nakamura, Tanabe & Katukawa, 1974) of the hen, and while levels of this steroid in the circulation have not been measured during the ovulatory cycle of the hen, it is known that serum androstenedione levels rise to a peak before the pre-ovulatory LH surge in women (Dupon, Hosseinian & Kim, 1973; Judd & Yen, 1973). Androstenedione could therefore play a part in stimulating LH secretion. However, as injections of between 0.1 and 1.0 mg androstenedione/kg did not increase plasma LH concentration in the hen (page 70) it is unlikely that androstenedione is the "active substance" proposed by Fraps (1955b). There is no evidence to suggest that testosterone can be converted to progesterone in the hen, though it is converted to oestradiol in the hen ovary (Nakamura, Tanabe & Katukawa, 1974). In the oestrogen-primed ovariectomized rat, an injection of testosterone propionate induces LH release (Brown-Grant, 1974) and this positive feedback effect of testosterone is interpreted as being the result of its conversion to oestrogen (Brown-Grant, 1974): this conversion is known to occur in the rat hypothalamus (Weisz & Gibbs, 1974). However, since both oestradiol-17β and oestrone were ineffective in stimulating LH secretion in the hen (page 71)it is unlikely that the positive feedback effects of testosterone suggested from this study are mediated via oestrogen. However, it is possible that other conversion products such as dihydrotestosterone and androstenediol (Nakamura & Tanabe, 1974) are involved.

Conclusion

This study has shown that testosterone, like progesterone, will induce an LH surge in the laying hen. Progesterone rapidly induces a release of LH when injected at all stages of the ovulatory cycle except

during the 4 h preceding an ovulation and exerts a similar effect in the oestrogen-progesterone primed ovariectomized hen. However, an LH response equivalent in magnitude only resulted from injections of testosterone at a time when a mature ovarian follicle was present. It is deduced that testosterone directly or indirectly stimulated the secretion of ovarian progesterone which then exerted a positive feedback effect on LH release.

Since the pre-ovulatory increase in testosterone secretion may precede that of progesterone, it is possible that before a normal ovulation rising levels of testosterone facilitate the release of other gonadal steroids, including oestrogen and progesterone. It is suggested that progesterone exerts a direct positive feedback effect at the hypothalamo-hypophyseal complex to cause the release of the pre-ovulatory LH surge, and that the oestrogen rise maintains a critical concentration of oestrogen in the blood required to prime the positive feedback centre to enable it to respond to progesterone.

It therefore appears that in the hen progesterone is the major steroid responsible for inducing the pre-ovulatory LH surge, whereas in mammals, oestrogen is more important in performing this role. In mammals, unlike birds, the embryo develops in the uterus and following ovulation the follicular wall persists as a corpus luteum which secretes progesterone. It is widely believed that the pre-ovulatory surge of LH secretion is involved in stimulating formation of the corpus luteum and thereby the secretion of progesterone. The two main functions of progesterone in the mammal are generally considered to be the maintenance of the uterus in a suitable condition for implantation of the embryo and to prevent the

development of further ova and ovulation by suppressing oestrogen secretion (Brown-Grant, 1969) and hence LH surges. However, if fertilization does not occur, the corpus luteum degenerates and the negative feedback effect of progesterone is lifted so that the ovulatory cycle can be repeated.

In the hen, the post-ovulatory follicle is rapidly resorbed (Deol, 1955; van Tienhoven, 1959) and although Deol (1955) did report the presence of "secretory luteal cells" in both pre- and post-ovulatory follicles, there is no evidence of a post-ovulatory rise of progesterone in the circulation (Furr et al., 1973a). Therefore in the aves, progesterone assumes a different role in the process of reproduction, notably that of triggering the events leading to ovulation. Progesterone may perform a similar role in amphibia since the injection of progesterone will induce ovulation in Xenopus laevis (Zwarenstein, 1937) or Rana pipiens (Langan, 1941; Wright, 1961; Masui, 1967). Testosterone also induces ovulation (Shapiro, 1936; Langan, 1941) while oestrogen is ineffective (Langan, 1941). In contrast to the hen, the presence of the anterior pituitary is not essential for this response in amphibians since progesterone can act directly on the follicle to induce ovulation (Wright, 1961).

It is therefore possible that in mammals, the divergence towards evolution of viviparity and the requirement of a hormone for maintaining pregnancy has taken progesterone from its original function as an ovulation-inducer and replaced it by oestrogen in this role.

C. THE ROLE OF THE YOLKY FOLLICLES IN MAINTAINING THE POSITIVE FEEDBACK RESPONSE IN THE HEN

Since the studies using ovariectomized hens suggest that an appropriate balance of progesterone to oestrogen in the blood is required to maintain the responsiveness of the positive feedback mechanism (pages 114 to 118), this balance is likely to be present only in intact hens when the ovary contains a full complement of follicles.

Progesterone failed to exert a positive feedback effect on LH release in moulting hens in which the ovary was regressed (page 91). It is apparent that the appropriate types of steroidogenic tissue must have been undifferentiated since although LH levels in moulting hens were similar to values obtained after laying had re-commenced, the secretion of appropriate quantities of oestrogen and progesterone required to prime the positive feedback mechanism were not stimulated. Imai, Tanaka & Nakajo (1972) similarly observed no difference between non-laying and laying hens in pituitary LH activity, as determined by the ovarian ascorbic acid depletion bioassay. However, these authors found that FSH activity of both the pituitary and plasma was higher in moulting than in laying hens, suggesting that the failure of follicular development was due to an imbalance of gonadotrophins (Imai et al., 1972).

The only consistent changes in LH secretion in a study of the moulting hen occurred at about 10 days before the onset of lay when plasma LH levels gradually rose to form a small peak at 5 days before the first oviposition (page 90). This was followed by a fall in

plasma LH levels when laying re-commenced. This small peak in LH secretion a few days before the first egg was laid after a period of moult was reminiscent of the pre-pubertal LH peak observed during the 8 to 12 weeks preceding the onset of lay in pullets (page 83). However, this rise in LH levels immediately prior to the onset of lay in moulting hens was both lower in magnitude and shorter in duration than the pre-pubertal LH peak. The reason for the LH peak is unclear, but may reflect the effects on LH secretion of changing ratios of steroids in the blood which might be expected a few days before the onset of lay when there is considerable follicular growth.

The inability of a progesterone injection to induce LH release in the non-laying hen was probably due to failure of response at the hypothalamic positive feedback receptor sites since the injection of progesterone fails to increase hypothalamic gonadotrophin releasing activity in non-laying hens, while increased activity is observed in layers (Tanaka, Kamiyoshi & Sakaida, 1974). A refractoriness of the hypothalamic positive feedback receptor sites to progesterone could result from changes in the ratio of progesterone to other steroids in the bloodstream; it has been shown that serum progesterone levels in turkeys fall as they go out of lay (Mashaly & Wentworth, 1974). It is considered that evidence for an alteration in the concentration of progesterone causing a refractoriness of the hypothalamus is provided by the observation that a decrease in the dose of progesterone injected during oestrogen-progesterone priming treatment in the ovariectomized hen reduced the positive feedback response to an intramuscular injection of 0.5 mg progesterone/kg (page 115).

It is apparent that the positive feedback effects of progesterone on LH secretion are not observed in the adult hen where the ovary does not contain large follicles. The LH response to progesterone was totally abolished within 48 h of removing all yolky follicles of more than 5 mm in diameter (page 93). Partial ovariectomy no doubt upsets the balance of steroid secretion by the remainder of the ovary, and shows that the positive feedback mechanism is desensitized within 48 h of the disruption of the pattern of gonadal steroid secretion characteristic of the laying hen. Since most of the ovarian oestrogen is synthesized in the small follicles of less than 5 mm diameter and ovarian stroma (Senior & Furr, 1975) and most of the progesterone is contained in the most mature ovarian follicle (Shahabi, Norton & Nalbandov, 1975), it seems likely that partial ovariectomy had decreased the progesterone : oestrogen ratio. As in the ovariectomized hens (page 115), a reduction in the concentration of progesterone to oestrogen in the blood would reduce or abolish the positive feedback response to a single injection of progesterone. A similar effect has been observed in the rat where ovariectomy at dioestrous diminished the positive feedback response in LH secretion to an injection of oestrogen at pre-oestrous (Tapper, Greig & Brown-Grant, 1974).

However, Kalra, Fawcett, Krulich & McCann, (1973) found that a priming injection of oestrogen shortly after ovariectomy of the rat at dioestrous enabled a single injection of progesterone to exert a positive feedback effect at pro-oestrous as in the normal cycle. Attempts to restore the positive feedback response in the partially

ovariectomized hen was unsuccessful and the normal LH response to a single injection of progesterone was not observed following priming injections of oestrogen, progesterone, or both oestrogen and progesterone (page 94).

It was noticeable that priming injections of oestrogen alone appeared to stimulate growth of the ovarian follicles in comparison with the effect of injections of progesterone alone (page 95). It is possible that the injected oestrogen stimulated the synthesis of yolk precursors by the liver (Vanstone, Dale, Oliver & Common, 1957). It has also been suggested that oestrogen increases the permeability of the follicular membrane (Clavert, 1958). If the same dose of oestrogen was combined with progesterone, no effect on the ovarian follicles was observed. It is possible that progesterone inhibited the oestrogen-induced increases in ovarian weight as has been suggested by similar observations in lizards (Jones, 1975).

It is interesting that the steroid replacement treatment was not adequate in partially ovariectomized hens, although oestrogen-progesterone priming does facilitate a positive feedback response in the completely ovariectomized hen. This difference may be explained by the fact that priming injections were given for 1 week in the completely ovariectomized hen, compared with 4 days after the operation of partial ovariectomy. Also steroid secretion from the remainder of the ovary would no doubt have modified the priming action of injected steroids.

D. DEVELOPMENT OF THE POSITIVE FEEDBACK RESPONSE DURING SEXUAL MATURATION

From observations in the partially ovariectomized hen, it might have been predicted that no positive feedback mechanism would have been demonstrable in the immature hen until just before the first ovulation. However, it was found that a positive feedback response developed at about 8 weeks before the first oviposition, though did not fully mature until a few days before this event.

A positive feedback action of progesterone on LH release was first observed when the pre-pubertal LH rise was beginning (see pages 80 & 84). Mammalian studies have suggested that the pre-pubertal rise in LH secretion is associated with a reduction in sensitivity of the negative feedback system to gonadal steroids (see page 139). If, in the immature pullet the negative feedback mechanism is very sensitive to gonadal steroids, it is likely that any tendency for progesterone to stimulate the release of LH in these birds by means of the positive feedback mechanism would be masked by a much stronger inhibitory effect caused by the response at the negative feedback site. This mechanism is functional in the sexually immature hen since ovariectomy at 12 weeks of age causes plasma LH levels to increase by 300 % within a week of operation (Sharp, 1973). Only when the negative feedback system becomes less sensitive to gonadal steroids, i.e. when pre-pubertal LH and steroid levels begin to rise, would it be possible for progesterone to stimulate the secretion of LH.

Since levels of gonadal steroids are rising when LH secretion can be first stimulated by a single injection of progesterone, they may

play a part in causing the maturation of the positive feedback mechanism. A major role for oestrogens in maturation and regulation of the onset of puberty in the rat has been suggested by several authors (Donovan & van der Werff ten Bosch, 1959; Ramirez & Sawyer, 1965; Smith & Davidson, 1968; Ying & Greep, 1971). In support of this concept, a single injection of oestrogen did not cause a positive feedback response in rats of less than 26 to 30 days of age (Ramirez & Sawyer, 1965; Ying & Greep, 1971; Caligaris, Astrada & Taleisnik, 1972). However, a prior injection of oestrogen facilitated the positive feedback effect of oestrogen in 22-day-old rats (Caligaris et al., 1972). These authors were unable to find evidence of a positive feedback response to oestrogen before this age even after steroid priming. In the rhesus monkey, the induction of an LH response by an injection of oestrogen was not apparent until after the onset of menarche, and it was not possible to advance the maturation of the positive feedback system with injections of oestrogen or progesterone (Dierschke et al., 1974). In contrast with the observations in the hen, the rat and the rhesus monkey, a positive feedback system was functional in 38-day-old ewes well before sexual maturity (Land, Thimonier & Pelletier, 1970).

The LH response to a single injection of progesterone in the hen varied during the seven stages of sexual maturation (pages 79 to 83). Although it has been shown that an injection of synthetic LH-RH (Etches & Cunningham, 1975) or ovine LH (Shahabi, Bahr & Nalbandov, 1975) stimulates the release of progesterone from the ovary, the induced progesterone surge subsides after 30 min and it was considered that the interval of 48 h between LH-RH and progesterone injections

in this study was sufficient to eliminate any effects resulting from the LH-RH secretion. Also, the characteristics of the LH responses to progesterone were related solely to the degree of sexual development and no differences were observed in the progesterone-induced LH surges between hens at the same stage of sexual development which had or had not received an earlier injection of LH-RH.

The amount of LH release in response to an injection of progesterone increased during the period when basal LH secretion was also increasing. However, as pituitary responsiveness to an injection of LH-RH was not increasing at the time of rising basal LH levels, then both the rising basal level of LH secretion and the increase in LH response to the injection of progesterone probably reflect increasing endogenous secretion of LH-RH, possibly accompanied by an increase in sensitivity of the positive feedback mechanism. In the rat, oestrogen receptors in the hypothalamus increase during sexual maturation (Kato, Atsumi & Inaba, 1974) to reach adult levels at about 28 days, and it is possible that the receptors in the brain of the immature hen increase at the time when the positive feedback system develops.

The maximal LH response to an injection of progesterone occurred at Stage III of sexual development when pre-pubertal LH levels had reached a peak of concentration (page 81). However, the response was short-lived compared with that at previous and succeeding stages of development (Fig. 21). This may reflect that there is a limited availability of releasable LH in the pituitary, or perhaps a negative feedback effect of a high blood level of LH.

After reaching a peak of basal secretion at about 4 weeks before the first ovulation, LH levels declined rapidly. This was associated

with a decrease in pituitary sensitivity to synthetic LH-RH, since the response obtained in hens with similar basal LH levels, but on the ascending slope of the pre-pubertal LH peak, was considerably greater. Also, the interval between the injection of LH-RH and peak LH values increased as the magnitude of the response diminished, probably as a result of steroid-dependent changes in the LH-releasing activity of the pituitary. As the pituitary became progressively insensitive to LH-RH, the LH response following an injection of progesterone also diminished until at Stage V of sexual development (page 82), when the ovary contained yolky follicles of up to 15 mm diameter, a single injection of 0.5 mg progesterone/kg was unable to stimulate the release of LH. However, although the maximal incremental change in LH levels following an injection of progesterone was reduced at Stages IV and V on the declining slope of the pre-pubertal LH peak, it was noticeable that the duration of the response was considerably greater than at Stage III of development where basal LH secretion had reached a peak (Fig. 21).

From these observations it is unlikely that the response to an injection of progesterone while basal LH levels were falling reflected entirely the changes in sensitivity of the pituitary. Other factors may also influence the amount of LH released by progesterone at various stages of sexual development. In particular, there may be variations in the amount of readily releasable LH in the pituitary, since there is a progressive increase in the size and potency of the gland during sexual maturation (Breneman, 1955). This may result from a reduction in the amount of LH released from the pituitary while synthesis of LH is unchanged.

Throughout the development of the positive feedback response, the latency period between the injection of progesterone and the time at which LH levels started to rise was gradually reduced from a period of about 2 h, when the response started to develop, to between 15 and 30 min at the last stage of development which was a few days before the onset of lay (Table 5). Since at all stages of development from 14 weeks of age until lay, at 22 to 28 weeks of age, an injection of LH-RH induced a rise in plasma LH levels within 2 min, it appears that the latency to LH response after an injection of progesterone was not due to changes in the latency to response of the pituitary. It probably reflected the degree of maturation of the neural processes in the positive feedback centre.

During the period of only a few days before the first ovulation, when there is rapid growth of large ovarian follicles, a maturational process occurs at the positive feedback centre. During this period, the amount of LH released in response to a single injection of progesterone increased although the sensitivity of the pituitary to LH-RH was lower than at previous stages of development (page 82). It is therefore apparent that the amount of LH-RH released after an injection of progesterone had increased dramatically. Since rapidly-growing, yolky ovarian follicles contain the highest concentration of progesterone in the ovary (Furr, 1969) it is possible that a changing ratio of progesterone to oestrogen in the blood brings about the final priming of the positive feedback mechanism.

This seems likely since where the concentration of progesterone in the circulation was increased by intramuscular injections of 0.5 mg progesterone/kg on 3 successive days, the response to a single injection

of progesterone was modified. At all stages of development, progesterone reduced the basal level of LH secretion and at Stages II and III of sexual development (page 88) reduced also the magnitude of the LH response to a single injection of progesterone. This may have been due to the steroid exerting a strong negative feedback effect on LH secretion which suppressed the positive feedback response. However, at Stages IV and V of development, where basal levels of LH normally fall as a result of a reduction in sensitivity of the pituitary to releasing factor, priming with progesterone significantly enhanced the LH response to a single injection of progesterone (page 89). Not only did progesterone priming increase the magnitude of the progesterone-induced LH surge, but it also significantly reduced the latency period to the onset of response (cf. Tables 7 and 5). This suggests that the gradual reduction in the latency period to response during development in unprimed pullets is the result of rising progesterone levels in the circulation. Since progesterone priming caused a reduction in basal LH secretion, the increased positive feedback response to progesterone at Stages IV and V of development is probably a reflection of the progesterone-induced maturation of the positive feedback site within the hypothalamus.

E. VARIATIONS IN THE POSITIVE FEEDBACK RESPONSE TO PROGESTERONE DURING THE OVULATORY CYCLE.

In the laying hen, the characteristics of the LH response were similar when progesterone was injected at most points of the ovulatory cycle (Fig. 14). However, when progesterone was injected during or in close proximity to the natural pre-ovulatory LH peak, the induced LH

surge was modified.

An injection of progesterone at 0 to 4 h before the pre-ovulatory surge of LH secretion was expected to occur, resulted in a suppressed LH response (page 64). This may be associated with increased secretion of oestradiol (Senior & Cunningham, 1974) and/or testosterone (Etches, 1974) which have been reported to occur during this period. During the 4 h preceding a pre-ovulatory LH surge, an increase in the blood concentration of oestrogens or androgens may exert a transitory negative feedback effect on the hypothalamo-hypophyseal complex; this may explain why a slight depression in plasma LH levels was found to occur just before the pre-ovulatory LH rise (page 50). However, injections of testosterone at a time when a mature follicle is present in the ovary have been shown to result in an LH surge (page 70) rather than exert a depressive effect on LH secretion. Also, pre-treatment of the oestrogen-progesterone primed ovariectomized hen with testosterone did not modify the LH response resulting from an injection of progesterone 2 h later (page 124). It is therefore more probable that increased levels of oestradiol in the blood shortly before the start of the pre-ovulatory LH peak modified the LH response to injections of progesterone. In support of this concept, pre-treatment of primed ovariectomized hens with oestrogen reduced the response to an injection of progesterone given 1 h later (page 123), although similar pre-treatment of the intact hen with oestrogen failed to modify the positive feedback response (page 72).

The LH response to injections of progesterone given when spontaneous pre-ovulatory LH levels were beginning to rise was more immediate and of greater magnitude than after the steroids were

administered during any other stage of the ovulatory cycle (page 65). In these circumstances the true magnitude of the induced LH release cannot be deduced since it was superimposed on the natural LH surge and inevitably contained a component of the latter. However, during this phase of the cycle, the hypothalamo-hypophysial complex may be "sensitized" to the positive feedback effect of progesterone, since LH levels started to increase within 15 min of the injection of progesterone, compared with a latency period of 15 to 45 min at other stages of the cycle. The immediate and rapid increase in LH levels was similar to the pattern of LH secretion following the injection of progesterone in laying hens or oestrogen-progesterone primed ovariectomized hens pre-treated about 45 min earlier with a dose of 0.05 or 0.1 mg progesterone/kg (pages 74 & 125). The dose of 0.05 mg progesterone/kg normally causes little or no increase in LH levels (pages 61 & 113) and it is likely therefore that the rising LH levels observed after the second progesterone injection were almost entirely a consequence of that injection. As the pituitary releases LH within 2 min of an injection of synthetic LH-RH in the intact and ovariectomized hen, it seems likely that the sensitizing effects of progesterone are directed at the positive feedback system within the hypothalamus by reducing the latency to the induced secretion of LH-RH, rather than by acting on the pituitary.

It is well-established that in mammals the pituitary becomes increasingly responsive to exogenous synthetic LH-RH during the period when pre-ovulatory LH levels are rising or about to rise. Mammals in which this has been demonstrated include the rat (Cooper, Fawcett & McCann, 1973; Aiyer, Fink & Greig, 1974; Martin, Tyrey, Everett &

Fellows, 1974b), ewe (Reeves, Arimura & Schally, 1971b), heifer (Kaltenbach, Dunn, Kiser, Corah, Akbar & Niswender, 1974) rhesus monkey (Krey, Butler, Weiss, Weick, Dierschke & Knobil, 1973; Ferin Warren, Dyrenfirth, Vande Wiele & White, 1974) and women (Yen, Vandenberg, Rebar & Ehara, 1972; Thomas, Cardon, Donnez & Ferin, 1973). In view of this and the enhanced LH response in the hen to an injection of progesterone, it is interesting to note that the incremental change in plasma LH levels in response to 20 µg synthetic LH-RH, intravenously injected when pre-ovulatory levels of LH were rising was similar to that observed at any other stage of the ovulatory cycle apart from the 3 h preceding ovulation, when the LH response is suppressed (Bonney, Cunningham & Furr, 1974). It might therefore be inferred that in the hen the pituitary was becoming more insensitive at this time and that the enhanced LH response observed on the ascending slope of a pre-ovulatory LH peak is due to a change in sensitivity at the positive feedback centre of the hypothalamus, rather than of the pituitary. However, since the LH response to injections of 20 µg LH-RH in the laying hen consists of a rise in concentration of less than 2 ng/ml (Bonney et al., 1974), it is possible that an increased response could not be easily demonstrated when measuring such small incremental changes in LH concentration.

In the present study, the effects of progesterone on the LH response to an injection of synthetic LH-RH were examined in the oestrogen-progesterone primed ovariectomized hen. The priming schedule, described on page 109, reduced LH levels to 6.3 ng/ml (page 128) and following the intravenous injection of 1 µg LH-RH/kg the concentration rose to 21.3 ng/ml (356 % increase). This magnitude of

LH response enabled changes in responsiveness of the pituitary to be more clearly observed. Forty-five minutes after an injection of 0.5 mg progesterone/kg, basal LH levels had risen in association with an increase in responsiveness of the pituitary to LH-RH. It is evident from these observations that a rise in circulating progesterone induces changes at the pituitary such as variations in the releasable pool of LH or an increase of LH synthesis. Progesterone may exert a direct effect on the pituitary, or alternatively, a rise in circulating progesterone may enhance endogenous LH discharge which may then increase pituitary responsiveness. An increase in responsiveness of the pituitary to LH-RH after prior treatment with LH-RH in the rat has been demonstrated by Aiyer, Chiappa, Fink & Greig, (1973). This would result in a greater release of LH in response to the injection of a constant dose of LH-RH, such as was observed in the present study.

However, this increased response was proportional to the basal level of LH secretion and if progesterone acted directly on the pituitary to sensitize it to LH-RH, it should be possible to demonstrate that progesterone can cause a rise in the percentage increase in LH secretion in response to an injection of a constant dose of LH-RH. This could not be demonstrated in the present study, although Arimura & Schally (1971) showed that pre-treatment with oestrogen enhanced the response of the rat pituitary to LH-RH without affecting basal LH concentration. The use of a dose of progesterone in the present study sufficiently low as not to raise basal LH secretion would perhaps have led to more definite conclusions on the effects of steroids in directly, or indirectly, increasing pituitary

sensitivity in the hen.

Since it has been shown in the hen that when pre-ovulatory LH levels are falling the pituitary becomes insensitive to synthetic LH-RH (Bonney et al., 1974), it is not surprising that progesterone was unable to stimulate LH secretion at this time. In view of the rapid fall in LH levels after a progesterone-induced surge of LH on the ascending slope of a pre-ovulatory LH peak (Fig. 13; page 65)it is possible that pituitary refractoriness may be brought about, at least in part, by previous exposure to increased circulating levels of progesterone. This does not exclude the possibility that the observed refractoriness may also be due partly to exposure to increased levels of other steroids, especially oestrogens, or to a rapid depletion of a readily releasable pool of LH.

The possibility that oestrogen causes pituitary refractoriness seems unlikely in view of the observation that an injection of progesterone on the declining slope of a progesterone-induced LH surge in the primed ovariectomized hen failed to stimulate LH secretion (page 126). Unless oestrogen was being secreted from some extra-gonadal source, such as the adrenal gland, it could not have caused the failure of progesterone to release LH. For this reason also, it is considered that androgens could not have been involved.

The possibility that increased circulating levels of progesterone are totally responsible for the pituitary refractoriness seems unlikely. Where laying hens were given two injections of progesterone at 2.5 h intervals it was found that the magnitude of the LH response to the second injection was related to whether or not the first injection had been given at a time when an LH surge had recently

been initiated (pages 75 to 77). If the first injection was given when LH levels were low, such as after the occurrence of an ovulation, this resulted in an LH surge, as judged by the increased LH levels 2.5 h later. A further injection of progesterone at this time failed to increase plasma LH levels. However, if the initial injection of progesterone was given on the declining slope of a natural pre-ovulatory LH peak, shortly before an ovulation, this failed to cause LH release, as judged by the low LH levels 2.5 h later. A further injection, however, caused a small increase in LH levels (pages 75 to 77). Since during either treatment the concentration of progesterone in the circulation would have been the same, then some factor other than the circulating level of progesterone must have been involved. These findings suggest that the availability of releasable LH in the pituitary is of considerable significance.

However, progesterone may also be partially involved since when the second injection caused a release of LH, it was only a diminished response compared with that observed after a single injection of the steroid at later than 4 h after an ovulation (cf. Figs. 19 & 13). Tanaka et al., (1974) found that gonadotrophin releasing activity of the hypothalamus was not changed by an injection of 2 mg progesterone /hen at 24 h before the second ovulation of a sequence (presumably at a time of elevated LH levels in the blood), whereas the same dose-level caused a significant increase at other times of the ovulatory cycle. It is possible that the hypothalamic positive feedback receptor sites become refractory to progesterone after a pre-ovulatory surge. Alternatively, the receptor sites may become saturated by the pre-ovulatory surge of progesterone, and unable to take up the injected

progesterone. If so, then little of the progesterone injected on the declining slope of the natural pre-ovulatory LH surge (page 75, Fig. 19d ii) would have been taken up at the receptor sites. This progesterone may then have decayed rapidly in the bloodstream and 2.5 h later the receptor sites may have been free again to take up progesterone from a second injection. In contrast, where an injection of progesterone after ovulation induced an LH surge (page 76, Fig. 19d i), the receptor sites would still have been saturated at the time of the second progesterone injection 2.5 h later, and no further stimulation of LH secretion would result.

It was noticeable that an injection of synthetic LH-RH on the declining slope of a progesterone-induced LH surge in the oestrogen-progesterone primed ovariectomized hen did cause a 100 % rise in LH levels (page 131). However, since there was a decrease in the responsiveness of the pituitary to LH-RH on the declining slope compared with uninjected oestrogen-progesterone primed ovariectomized hens (108 % versus 356 % increase) it confirms the findings of Bonney et al., (1974) that the pituitary becomes insensitive at the stage of the cycle where pre-ovulatory LH levels are falling. Although there was a decrease in pituitary sensitivity at this time, circulating LH levels were still high, and it is apparent that high levels of LH secretion must have been maintained by a rate of endogenous LH-RH discharge which was greater than that occurring before a progesterone-induced LH surge. It is deduced that progesterone not only stimulates LH secretion by enhancing pituitary responsiveness to LH-RH but also raises the rate of endogenous LH-RH secretion.

Since an injection of 1 μg LH-RH/kg on the declining slope of

a progesterone-induced LH surge stimulated LH secretion in the ovariectomized hen, it suggests that a single injection of 0.5 mg progesterone/kg failed to deplete the releasable LH from the pituitary. However, a further injection of 0.5 mg progesterone/kg on the declining slope of the progesterone-induced LH surge failed to stimulate LH secretion, and in view of the observations of Tanaka et al., (1974) it is possible that the amount of LH-RH released in response to the second injection of progesterone was less than that released by the first injection. Alternatively, the same quantity of LH-RH may have been released by both progesterone injections, but it is possible that the releasable pituitary LH does not exist as a discrete "pool" but in a form which may become progressively less releasable as the pituitary stores become depleted.

For many mammals, it is suggested that the period after ovulation during which LH surges cannot again be initiated by the injection of oestrogen is controlled by the secretion of progesterone from the corpus luteum, which by a negative feedback effect suppresses gonadotrophin secretion (Brown-Grant, 1969; Barraclough, 1973). Only when this suppression is lifted by a fall in circulating progesterone levels can LH surges again be initiated. In the hen, the structure homologous to the corpus luteum is the post-ovulatory follicle, and although the follicle contains progesterone (Furr, 1969) there is no evidence that it actively secretes this steroid. In contrast to mammals, a small surge of LH can again be initiated by an injection of progesterone as soon as the concentration of LH in the circulation drops to basal level, i.e. at about the time of ovulation. A full LH response resulted from injections of progesterone at between 2 and

4 h after ovulation, indicating that a period of 2 to 4 h is required for either the pituitary to synthesize sufficient quantities of releasable LH or for a recovery in the sensitivity of the hypothalamic positive feedback mechanism.

F. OTHER ROLES FOR PROGESTERONE IN THE CONTROL OF THE OVULATORY CYCLE

1. The timing of oviposition

It seems likely from the observations in this study that the naturally occurring pre-ovulatory rise in the concentration of progesterone in the plasma has a role to play in the regulation of both ovulation and oviposition.

A close relationship between the times of oviposition of one egg and ovulation of the next was first reported by Warren & Scott (1935). They observed that oviposition generally takes place 14 to 75 min before ovulation except at the end of a sequence, which suggests that there is a causal relationship between these two events. Since oviposition generally precedes ovulation, it could be assumed that in mid-sequence ovulation is dependent on the process of oviposition. However, this has not been supported experimentally. Thus, where oviducal eggs were prematurely expelled by manual crushing (Warren & Scott, 1935) ovulations were not advanced.

However, there is evidence that the timing of oviposition is determined, partially at least, by the ovulatory process. Fraps (1955a) suggested that the time of oviposition is dependent principally on two factors. The first is the integrity of the post-ovulatory follicle which influenced the time of oviposition of the egg to which it gave rise. This was deduced from the observation that if

the post-ovulatory follicle was removed oviposition was delayed, whereas if it was left intact and the oldest maturing follicle removed, oviposition occurred at the predicted time (Rothchild & Fraps, 1944). The second factor is associated with the ovulation of the next egg in the sequence. Thus when an ovulation was induced prematurely by injecting LH, oviposition of the egg in the oviduct at the time of hormone injection was also premature (Fraps, 1942). As oviposition precedes ovulation (Warren & Scott, 1935), the factor associated with the ovulatory process which controls the time of oviposition must be effective during the period preceding ovulation.

At 9 to 4 h before ovulation there are increases in the plasma concentration of androgens, oestrogens and progesterone (see pages 23 to 25). Previous studies have shown that the time of a predicted oviposition can be altered by an injection of progesterone (Brard, 1961; Hawkins, Heald, Swain & Taylor, 1970) or testosterone (Hawkins et al., 1970), though the quantitative effects of these steroids on the timing of oviposition were not investigated.

This study has shown that either testosterone or progesterone can delay and, in the case of progesterone, also advance the timing of oviposition. However, the delaying actions of these two steroids differed. Doses of between 0.5 and 2.0 mg testosterone/kg injected between 0 and 9 h after a predicted ovulation delayed oviposition of that egg by between 2 and 48 h and appeared to delay or prevent the next expected ovulation. Also, when injections were given about 24 h after the last ovulation of a sequence, at a time when progesterone injections normally cause premature ovulation of the first egg of a sequence and thereby extend the length of the sequence by one egg

(Neher & Fraps, 1950; and page 101), testosterone usually delayed this ovulation or prevented it from taking place. Since injections of testosterone at this time stimulated an LH surge (page 70), probably by causing the release of endogenous ovarian progesterone (page 162), it seems that the testosterone injection prevented the ovulation-inducing effects of the LH surge, or it is possible that ovulation was induced but that testosterone affected oviduct motility and thereby delayed oviposition of that egg.

In contrast to the effects of testosterone, when progesterone was administered between 0 and 6 h after ovulation, the interval between injection and oviposition was constant, and related to the dose injected, i.e. 26.0 $\pm$ 0.35 (S.E.M.) h after 0.1 mg progesterone/kg and 30.6 $\pm$ 0.22 h after 0.5 mg progesterone/kg (page 97). Since the delayed oviposition did not occur simultaneously with ovulation, it seems likely that the exogenously administered progesterone was controlling the time at which oviposition occurred. The interval of about 31 h between injection of 0.5 mg progesterone/kg and oviposition is similar to that which elapses between the maximum of the pre-ovulatory LH surge and the resulting oviposition (page 51). This interval is constant and demonstrates that the pattern of distribution of eggs laid throughout the day (Fig. 10) is dependent on the time of the LH surge which initiated the ovulation of those eggs. It is possible that the delaying effect of progesterone on oviposition described above is mediated indirectly by causing an increase in plasma LH concentration. This is supported by the observation that the pre-ovulatory rise in the blood concentration of progesterone is concurrent with a rise in the level of LH (Furr et al., 1973a)and the

injection of progesterone at any stage of the ovulatory cycle, except when pre-ovulatory LH levels are falling, will stimulate LH secretion (pages 62 to 67). However, injections of 0.5 and 0.1 mg progesterone /kg given between 0 and 6 h after ovulation exerted significantly different delaying effects on the timing of oviposition while injections of such doses of the steroid resulted in similar incremental changes in plasma LH levels (page 61). It therefore seems likely that the timing of oviposition is influenced by an increase in the plasma level of progesterone rather than of LH.

When injections of progesterone were given between 6 and 15 h after ovulation, ovipositions were often delayed until the time of the next ovulation, which was generally delayed by one day (Fig. 25c). In these circumstances the interval between progesterone injection and the delayed oviposition was variable, ranging from 35.0 h to 67.0 h. It is therefore suggested that the injection of progesterone at 0 to 6 h after ovulation over-rode the effects of the preceding pre-ovulatory progesterone surge and oviposition was synchronized to the injection. However, where progesterone was injected 6 to 15 h after ovulation, oviposition of that ovulated egg was not regulated by the exogenous progesterone but by the next naturally occurring pre-ovulatory surge of progesterone. This seems likely, since injections of progesterone given 12 to 9 h before a predicted ovulation caused premature oviposition (Fig. 25d) in association with premature ovulation. It therefore appears that once an egg has been in the oviduct for more than 15 to 18 h, an increased level of progesterone in the circulation will cause it to be oviposited. Since a naturally occurring ovulation is always preceded by an increase in the plasma

level of progesterone, this mechanism ensures that an oviducal egg is expelled at about the time of ovulation, so that the oviduct seldom contains 2 eggs.

Though this study has revealed a pronounced effect of progesterone on the timing of oviposition, the mechanism whereby this is brought about remains to be discovered. The pre-ovulatory rise in blood progesterone levels may not be the only factor involved in the timing of oviposition: the integrity of the post-ovulatory follicle appears to be of importance (Rothchild & Fraps, 1944; Tanaka & Nakada, 1974), though its significance is not understood. Also the hormones of the posterior pituitary gland may be involved in expelling the oviducal egg (Sturkie & Lin, 1966; Rzasa & Ewy, 1970) though the actions of these hormones are immediate since an injection of vasotocin will cause premature oviposition of an egg within 2 minutes. However, it appears that the approximate timing of this event is regulated beforehand and this study suggests that the single surge of progesterone which occurs 7 to 4 h before an ovulation influences the timing of both the oviposition of the egg resulting from that ovulation and the oviposition of the previously ovulated egg.

2. Possible involvement of progesterone in the termination of an egg-laying sequence.

A pre-ovulatory LH surge associated with the first ovulation of a sequence occurs shortly after the onset of darkness under a photoschedule of 14 h light : 10 h darkness (page 50). It is therefore apparent that for the hens used in this study the onset of darkness corresponds with the start of the "open period" of the ovulatory cycle

during which a pre-ovulatory surge of LH can occur (Fraps, 1954). The "open period" of the cycle in this study lasted for about 9 to 10 h and most of the LH surges in different hens associated with a final ovulation of a sequence were initiated shortly before or after the onset of light. During the remaining 14 h "closed period" spontaneously generated pre-ovulatory surges of LH were found not to occur.

With regard to the phenomenon of the "open period", a parallel can be drawn between the hen and small rodents where spontaneous or oestrogen-induced LH discharges are restricted to a limited period of the day, termed the "critical period" (Schwartz, 1969, 1973; Everett, 1972; Norman, Blake & Sawyer, 1973). However, Fraps suggested that the "open period" in the hen and the "critical period" in rodents were not the same (Fraps, 1965) since an injection of progesterone can force the ovulation of a mature follicle in the hen, and therefore release LH at times outside the "open period" (Fraps & Dury, 1943; Rothchild & Fraps, 1949b; Neher & Fraps, 1950).

In the present study it was shown (page 67) that in the hen, as in the ewe (Jackson & Thurman, 1974) and rhesus monkey (Karsch et al., 1973b), LH secretion can be stimulated by an exogenous gonadal steroid at any time of day, and therefore at any time during the "closed period" of the hen's ovulatory cycle, provided it is not injected when pre-ovulatory LH levels are falling. According to Fraps (1954, 1961, 1965) natural ovulations do not occur during the "closed period" because the neural component of the LH release mechanism shows a decreased sensitivity to the positive feedback effects of ovarian steroids (see page 18). If Fraps is correct then the dose of

progesterone used in this study to stimulate LH secretion during the ovulatory cycle (0.5 mg/kg; pages 62 to 67) must have resulted in circulating levels of the steroid sufficiently in excess of normally occurring peak pre-ovulatory levels to overcome a high threshold of sensitivity to progesterone. In view of the observations of Etches & Cunningham (1975), discussed on page 150 , this seems to have been likely. However, since the variations in the LH response to an injection of progesterone during the ovulatory cycle were related to the occurrence of a pre-ovulatory LH surge, these observations do not provide any direct evidence to support the view that there is a diurnal rhythm of sensitivity to progesterone during the "open period" of the ovulatory cycle.

The reason for the restriction on the occurrence of pre-ovulatory LH surges to the "open period" is therefore not clear. It is not due to a failure of the positive feedback mechanism controlling LH release, nor is it due to the absence of an ovulable follicle since the first ovum of a sequence can be prematurely ovulated by injections of progesterone during the "closed period" of the cycle. Since the LH surge associated with the first ovulation of a sequence occurs at the onset of darkness under a 14 h light : 10 h darkness photoschedule, and yet that follicle can be readily ovulated several hours earlier by the injection of progesterone or LH, then it appears that some signal associated with the onset of darkness may trigger a surge in the secretion of gonadal steroids. In view of the observation that a small rise in LH levels usually occurs at the onset of darkness (page 50) it is tempting to suggest that this small LH rise triggers the secretion of steroids from the ovary. This possibility is supported

by the observation that the injection of synthetic LH-RH (Etches & Cunningham, 1975) or ovine LH (Shahabi, Bahr & Nalbandov, 1975) induces the secretion of progesterone from the ovary. The small LH rise at the onset of darkness occurred whether or not it was followed by a pre-ovulatory LH surge, suggesting that it was not dependant on the ovulatory process. It is possible that the reduction in activity associated with the onset of darkness was responsible directly or indirectly for causing a small increase in LH secretion. It has been shown that where a delay to the onset of darkness was combined with enforced wakefulness the first ovulation of a sequence was delayed (Bastian & Zarrow, 1955) suggesting that there had also been a delay in the pre-ovulatory release of gonadal steroids and LH.

Although it has been shown that steroidogenesis increases rapidly at about 19 h after a previous ovulation, at the same time as the secretion rate of gonadal steroids rises (Shahabi, Norton & Nalbandov, 1975), the relationship between the synthesis of progesterone in the follicle destined to ovulate as the first of a sequence and the actual pre-ovulatory release of that steroid has not been determined. It is possible that the increase in steroidogenesis may have occurred about 19 h after the previous ovulation, but that during the "closed period" of the ovulatory cycle, the ovary may not receive a "signal" to trigger the release of the quantities of gonadal steroids needed to stimulate the ovulatory surge of LH. The nature of this "signal" is unclear. Since LH peaks are normally only initiated during or shortly after the period of darkness, it is possible that there exists a diurnal rhythm of gonadotrophin secretion with higher secretion rates during darkness than during the day. The observations

in this study do not suggest a diurnal rhythm of LH secretion, though it is possible that the rate of FSH secretion is related to the time of day.

It is also possible that the follicle may not have been sufficiently mature to be able to respond to this signal and the increase in steroidogenesis normally occurring at 19 h after the previous ovulation had been delayed until the onset of the dark period when the small rise in LH levels could trigger the synthesis and release of gonadal steroids.

While observing the effects of progesterone injections on the timing of oviposition, it was noticeable that injections between 12 and 9 h before a predicted ovulation often advanced that ovulation and the time of oviposition of the previously ovulated egg by 2 to 3 h. However, injections 27 to 12 h before a predicted ovulation delayed the next expected ovulation usually by 1 day and occasionally by up to 3 days. A delaying effect as opposed to a blocking effect was observed also by Fraps (1954) who delayed ovulation by 1 day after injecting hens with large doses of oestradiol benzoate. An injection of progesterone generally delayed ovulation until the time of day at which the first ovulation of a sequence would be expected to occur (page 99). It is possible that since the injection of progesterone in this study induced a release of LH at any time of the cycle apart from during the 4 h preceding ovulation, then the endogenous LH peak may have caused the follicle to synthesize and release progesterone. In this case, the follicle may not have been able to synthesize more progesterone until the "open period" had ended, and the synthesis and release of gonadal steroids would then be delayed until the start of the next "open period".

Therefore the effects of a surge of progesterone in the blood between 0 and 6 h after the occurrence of an ovulation were to delay oviposition of that egg until late in the afternoon, at a time when the final oviposition of a sequence normally occurs, and to delay the next ovulation by nearly 24 h until early on the second day after injection, i.e. at a time of day when the first ovulation of a sequence would normally be expected to occur (Fig. 25). It is therefore apparent that a rise in the circulating concentration of progesterone shortly after the occurrence of a pre-ovulatory surge of progesterone can induce effects comparable with those occurring at the end and beginning of a normal sequence.

When examining the effects of an injection of progesterone on LH release during the ovulatory cycle, it was observed that circulating LH levels were high on the two occasions where progesterone had been injected shortly after the penultimate oviposition of a sequence, (page 66). This seems to indicate that the final ovulation of a sequence may occur later than the estimated 14 to 75 min after that oviposition (Warren & Scott, 1935). If this were so, it is possible that the pre-ovulatory progesterone surge associated with that ovulation occurred later in relation to the time of the accompanying oviposition than is normal during the remainder of the sequence. This may be indicated by the observation that the peak progesterone levels occur later in relation to the time of an oviposition and predicted ovulation in mid-sequence than in relation to the first ovulation of a sequence (Haynes, Cooper & Kay, 1973). A late progesterone surge may therefore produce the same delaying effects as an injection of progesterone shortly after ovulation in this study. It may delay an

ovulation by slowing down the rate of maturation of the largest ovarian follicle so that it does not acquire the ability to synthesize and/or release ovulable quantities of progesterone until after the end of the "open period". The mature follicle would then have to wait until a "trigger" associated the start of the "open period" at the onset of darkness (perhaps, the small rise in LH levels observed at the time) would set in train the motions leading to ovulation.

Whenever ovulation was either advanced or delayed by injections of progesterone, the subsequent ovulations in the sequence were also advanced or delayed. The resulting eggs in the induced sequence were laid at intervals characteristic of the normal pattern for the hen (Table 12). In view of this, it appears that alterations in the integrity of the most mature follicle, caused by progesterone, influence the development of the remainder of the follicular hierarchy. Opel and Nalbandov (1961) have suggested that follicular gradation is maintained by a regulation of the amount of gonadotrophin reaching the developing follicles and it is possible that rising levels of gonadal steroids may regulate the secretion of FSH or the uptake of that gonadotrophin by the follicles.

VI. A HYPOTHESIS FOR THE REGULATION OF THE OVULATORY CYCLE OF THE HEN.

In previous hypotheses proposed to explain the regulation of the hen's ovulatory cycle (see pages 15 to 19) a main drawback has been the inability of the hypothesis to explain the reason why mature follicles do not accumulate as a result of the delay in ovulation

which terminates a sequence of eggs. The observations of the present study may contribute towards an understanding of that problem.

For the hens used in this study, the "open period" for the induction of a pre-ovulatory LH surge is about a 10 h period of the 24 h day commencing shortly after the onset of darkness within a photoschedule of 14 h light : 10 h darkness (lights on 07:00 h to 21:00 h). Thus the LH surge associated with the first ovulation of a sequence was found to start soon after the lights had gone out. Of the three major types of gonadal steroids which are released 7 to 4 h before ovulation, i.e. androgens, oestrogens and progesterone, this study indicates that progesterone is directly responsible for inducing the LH surge, although testosterone may be involved, possibly by causing the release of ovarian progesterone through either direct action on the ovary or indirectly by the involvement of the hypothalamo-hypophyseal complex. Oestrogen is mainly secreted from the small growing follicles (Shahabi, Norton & Nalbandov, 1975; Senior & Cunningham, 1975) and probably the pre-ovulatory peak of secretion contributes to maintaining a critical level of oestrogen in the blood required to maintain the sensitivity of the positive feedback mechanism. However, an increase in the blood level of progesterone appears to cause directly a surge of LH secretion starting within 15 to 45 minutes. This observed response is similar to what was reported by Furr et al., (1973a) before a natural ovulation.

It appears that progesterone is responsible for not only causing ovulation but also for regulating the timing of oviposition of the egg to which it gave rise. Also, except at the beginning and end of a sequence, progesterone appears to determine the time of

oviposition of the previously ovulated egg, so that there is a close temporal relationship between the events of oviposition and ovulation which ensures that only 1 egg is in the shell gland of the oviduct at any time. A disruption of this mechanism could lead to a newly ovulated egg entering the shell gland before the shelled egg is discharged leading to the simultaneous expulsion of both eggs.

An asynchrony between the approximate 26 h cycle of ovum maturation and the 24 h day results in gonadal steroid and LH peaks occurring slightly later each night as the sequence progresses. This is reflected in the oviposition patterns of hens shown in Table 12 and Fig. 25. In Fig. 10 the peak period of egg laying during the day was observed between 09:00 h and 10:00 h, about 2 to 3 h after the onset of the light period. There is then a drop in the number of eggs laid in the succeeding hours until a small increase again at about 15:00 to 18:00 h. This small increase reflects the last oviposition of a sequence which occurs at a greater time interval after the previous oviposition than is observed at any other time during the sequence. It could be suggested that because the timing of the final oviposition of a sequence is dependent on the progesterone surge initiating the ovulation of that egg and not on the influence of the progesterone peak associated with the next occurring ovulation, then it spends more time in the oviduct than do previous eggs of a sequence. This seems unlikely, however, since the interval between a progesterone injection 0 to 6 h after ovulation and the resulting delayed oviposition was 31 h and this oviposition did not occur in association with an ovulation. This is similar to the interval between the LH surge and ovipositions of eggs in mid-sequence. Alternatively, if the interval between the pre-

ovulatory progesterone and LH peaks and the oviposition of the egg resulting from the peak is constant for all eggs in a sequence, it is possible that the final ovulation of a sequence occurs more than 14 to 75 min (Warren & Scott, 1935) after the occurrence of the penultimate oviposition. In view of the high LH levels observed up to 2 h after the penultimate oviposition of a sequence had already occurred, it is therefore likely that the last egg of a sequence reaches maturity more slowly than the previous eggs. This is consistent with the observations of Lacassagne (1960) that the maturation of a follicle and ovulation do not occur simultaneously and that the period of yolk deposition decreases in eggs towards the end of a sequence (Lacassagne, 1960; Bacon & Skala, 1968). An increasing immaturity of the later ovarian follicles of a sequence at the time of ovulation is also suggested by the report that where egg sequences are extended by the induction of ovulation on the usual day of lapse, the yolks of the hormone-induced eggs become progressively smaller (Neher & Fraps, 1950).

If the succeeding ovarian follicle was maturing at an even slower rate, a late surge of progesterone preceding the final ovulation of a sequence may be able to affect the maturing follicle at a stage of development when it is very sensitive to progesterone and the development of the follicle may then be retarded even more than the previous one. It may not be able to synthesize or release ovulable quantities of gonadal steroids until the "closed period" has begun.

The "closed period" for hens used in this study started at about the time the lights came on (about 07:00 h) though it is unlikely that the onset of the light period determines the ending of the "open period" since in one hen in which LH levels were measured during 24 h,

pre-ovulatory LH levels associated with the final ovulation of a sequence must have begun to rise at least 2 h after the lights had come on (page 50). It is therefore unlikely that light is the factor preventing the initiation of LH surges. It is also improbable that the maturation of an ovarian follicle could be delayed by progesterone until exactly the onset of the "open period" (i.e. onset of darkness). It is more probable that the development of the ovarian follicle is delayed to the extent that it matures during the "closed period" of the cycle during which time it would not be stimulated to synthesize and/or release ovulable quantities of gonadal steroids. That follicle would, during the "closed period" be sufficiently mature to be ovulated by exogenous progesterone or LH, but would require a stimulus associated with the onset of the "open period" in order to synthesize and/or release steroids. This stimulus is possibly the small peak of LH secretion occurring at that time.

It is unlikely that only the ovarian follicle which normally becomes the first ovulation of a sequence could be affected in this way by progesterone. The delaying effect is probably progressive and is experienced to a lesser extent by other eggs of a sequence. The delay in the final maturation of the most mature follicle which results in a termination of a sequence may retard the development of the remainder of the large ovarian follicles so that the normal follicular hierarchy is maintained.

A regulation of the rate of development of the growing follicles is necessary since the follicles at various stages of development are discriminatory in their synthesis and secretion of gonadal steroids (Senior & Furr, 1975; Shahabi, Norton & Nalbandov,

1975). As discussed on page 156, an interruption in the normal ratio of progesterone to oestrogen in the blood which might occur if the follicular hierarchy was disrupted would prevent the normal functioning of the positive feedback mechanism and thereby result in a failure of ovulation.

VII. CONCLUSIONS

1. This study has indicated that progesterone plays a major role in regulating the ovulatory cycle of the hen.
2. In view of the observation that progesterone exerts a direct positive feedback effect on the secretion of LH, this strongly suggests that it normally regulates cyclic LH secretion.
3. Apart from its involvement in the control of ovulation, the pre-ovulatory progesterone surge appears to control the timing of oviposition in such a manner that, except at the end of a sequence, the events of oviposition and ovulation occur in close proximity to each other. Also, this mechanism normally prevents two eggs from being in the oviduct at the same time. However, a common occurrence at the onset of egg production is the oviposition of two eggs during one day. Usually one of these eggs is shell-less or soft-shelled, which probably results from a newly ovulated egg entering the shell-gland before the previously ovulated egg has been expelled. Both eggs would then be expelled concurrently. This disruption in the timing of oviposition and ovulation points to a failure in the processes by which progesterone controls these events.
4. Infrequent oviposition is common during the early period of

production. Since an ovulation is delayed or blocked by an injection of progesterone in mid-sequence at any time of the ovulatory cycle, apart from during the 3 h preceding the normal pre-ovulatory release of that steroid, it is possible that this infrequent laying is the result of premature or delayed pre-ovulatory release of progesterone preventing or delaying the next expected ovulation. In the same way, a comparatively late progesterone surge at the end of a normal sequence may delay the maturation of the next largest follicle until outside the open period, so terminating the sequence.

5. The laying efficiency of a hen may be determined during its growth and development since this study has revealed some evidence that both the magnitude and duration of the pre-pubertal LH surge, and the LH response to an injection of progesterone in the immature hen, are related to the number of eggs laid during the first 6 months of production.

6. An investigation of irregularities in the way in which progesterone controls the processes of LH release, ovulation and oviposition could be of value in identifying some of the inherent factors associated with laying efficiency in poultry.

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VARIATIONS IN PLASMA LH LEVELS DURING THE OVULATORY CYCLE OF THE HEN, *GALLUS DOMESTICUS*

SUSAN C. WILSON AND P. J. SHARP

Agricultural Research Council Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS, Scotland

(*Received 9th July* 1973)

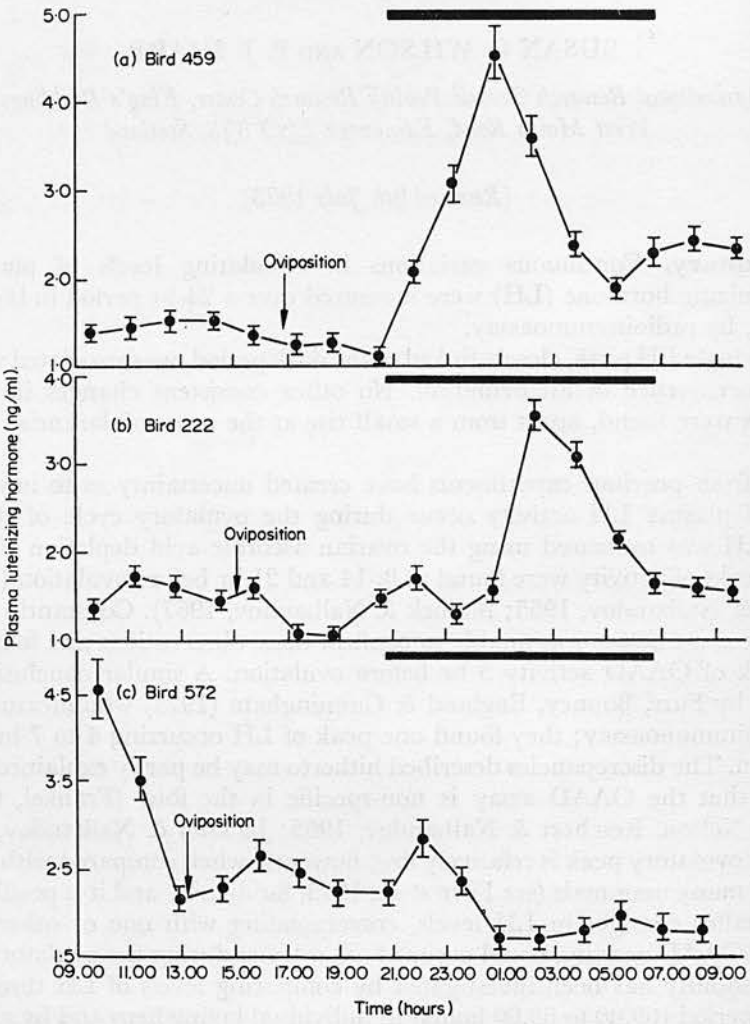
Summary. Continuous variations in circulating levels of plasma luteinizing hormone (LH) were measured over a 24-hr period in laying hens, by radioimmunoassay.

A single LH peak, closely linked to the dark period, was associated with the occurrence of an ovulation. No other consistent changes in LH levels were found, apart from a small rise at the onset of darkness.

Results from previous experiments have created uncertainty as to how many peaks of plasma LH activity occur during the ovulatory cycle of the hen. When LH was measured using the ovarian ascorbic acid depletion (OAAD) assay, peaks of activity were found at 8, 14 and 21 hr before ovulation (Nelson, Norton & Nalbandov, 1965; Bullock & Nalbandov, 1967). Constantin (1969), using the same assay, was unable to confirm these observations and found only one peak of OAAD activity 5 hr before ovulation. A similar conclusion was reached by Furr, Bonney, England & Cunningham (1973) who measured LH by radioimmunoassay; they found one peak of LH occurring 4 to 7 hr before ovulation. The discrepancies described hitherto may be partly explained on the ground that the OAAD assay is non-specific in the fowl (Frankel, Gibson, Graber, Nelson, Reichert & Nalbandov, 1965; Jackson & Nalbandov, 1969). The LH ovulatory peak is relatively low, however, when compared with similar peaks in many mammals (see Furr *et al.*, 1973, for details) and it is possible that even smaller changes in LH levels, corresponding with one or other of the peaks of OAAD activity, could occur at other times during the ovulatory cycle. This possibility has been investigated by comparing levels of LH throughout a 24-hr period (09.00 to 09.00 hours) in individual laying hens and by attempting to improve the precision of the radioimmunoassay.

The birds were caged individually, allowed free access to food and water and exposed to 14 hr light/10 hr darkness (lights off from 20·45 to 06·45 hours). The times of oviposition were recorded automatically. Blood samples (0·5 ml) were taken by venepuncture from the wing veins of twenty-three laying 12- to 18-month-old hens (thirteen derived from a Thornber 909 strain and ten from a Shaver strain) at 1½-hr intervals, and plasma LH levels measured using the radioimmunoassay developed for fowl LH by Follett, Scanes & Cunningham

(1972). The standard and ^{125}I-labelled tracer was a preparation of fowl LH (fraction AE1) purified to minimize TSH activity (Scanes & Follett, 1972). Each plasma sample was assayed in duplicate at three dilutions and the potency estimates, together with their 95% confidence limits, were computed using a modified version of the programme developed by Rodbard & Lewald (1970).



TEXT-FIG. 1. Variations in plasma LH levels in individual laying hens during a 24-hr period. The vertical bars represent 95% confidence limits. The black bars represent the dark period.

In the eight assays required to complete this study, the minimum detectable level of LH was 0·041 ng/ml$\pm$0·010 S.D. and the potency of a control plasma, included in each assay, was 10·61 ng/ml$\pm$0·23 S.D. Ovipositions within 36 hr of taking the last blood sample were used as evidence of an ovulation having occurred during or shortly after the 24-hr study period.

One major peak of LH was found in all the eleven hens (six Thornbers, five Shavers) which ovulated during this period but no such peaks were found in the twelve birds which did not ovulate. The peaks had maximum values ranging from 3·00 to 4·54 ng/ml (mean = 4·17 ng/ml ± 0·75 S.D.) and took between 4 and 7 hr (mean = 5·8 hr ± 1·0 S.D.) to rise from and return to baseline levels. A slight decrease in LH immediately preceding the peak was evident in all but one case. There was no significant difference in baseline levels (combined mean = 1·82 ng/ml ± 0·20 S.D.) between birds with or without an ovulation.

The occurrence of an LH peak was closely linked to the dark period. In no case did LH levels start to rise before the lights went out or more than 1 hr after the lights came on (see Text-figs 1a to c). As shown in Table 1, a time relationship of 31·5 to 34·0 hr (mean 32·5 hr ± 0·8 S.D.) was established between maximum LH levels and the resulting oviposition. There were insufficient data

Table 1. The time relationship between the LH ovulatory peak and oviposition in the hen, *Gallus domesticus*: lights went off at 20.45 and on at 06.45 hours

Bird no.	*Maximum LH peak (time of day)*	*Oviposition of egg ovulated by LH peak (time of day)*	**Interval (hr) between LH peak and resulting oviposition*	**Interval (hr) between LH peak and oviposition of previously ovulated egg*
459 (Shaver)	00.30	09.49	33½	No egg laid
8804 (Thornber)	01.00	11.00	34	No egg laid
328 (Shaver)	01.00	08.13	31½	No egg laid
222 (Shaver)	01.30	09.21	32	No egg laid
9579 (Thornber)	02.00	09.14	31½	No egg laid
9170 (Thornber)	02.30	10.51	32½	No egg laid
8413 (Thornber)	06.30	15.43	33	5
9405 (Thornber)	08.00	16.57	33	4
8297 (Thornber)	09.00	18.00	33	4
571 (Shaver)	09.30	18.08	32½	10
572 (Shaver)	10.00	17.32	31½	4½

*To the nearest ½ hr.

to establish a similar relationship between the LH peak and the oviposition of the egg previously ovulated (Table 1).

In sixteen birds, LH values showed a small but significant rise at the onset of the dark period, irrespective of whether they were due to ovulate later that day, and in nine cases this rise took the form of a minor peak. In three other birds showing an early ovulatory peak of LH, the rise may have been obscured and, of the twenty-three hens studied, only four failed to show an elevation at that time. No other consistent changes in circulating levels of LH were detected. In particular, the fifteen ovipositions which occurred during the 24-hr study period were not associated with any consistent change in plasma LH levels.

In conclusion, these data support the view that there is only one ovulatory peak of LH in the ovulatory cycle of the hen. Other small rises can occur, notably immediately after the onset of darkness, but there is no evidence that they are related directly to the ovulatory process.

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EPISODIC RELEASE OF LUTEINIZING HORMONE IN THE DOMESTIC FOWL

SUSAN C. WILSON AND P. J. SHARP

Agricultural Research Council's Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh, EH9 3JS

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SUMMARY

Plasma luteinizing hormone (LH) levels were measured by radioimmunoassay in serial samples taken from intact adult cockerels, gonadectomized fowl of both sexes and laying hens. By sampling at 30 and 10 min intervals, it was shown that in cockerels LH is released episodically. Each secretory episode lasted 90–120 min and was characterized by a 100–200% rise in LH levels over a period of 10–15 min followed by a more gradual, exponential decline. Mean plasma LH levels were found to be depressed in cockerels as a result of the handling associated with taking blood samples; in cases where LH levels were most depressed, an episodic pattern of secretion could not be demonstrated.

In the gonadectomized fowl, an episodic pattern of LH secretion was shown when blood samples were taken at 10 or 5 min intervals. During each secretory episode, which lasted 20–45 min, LH levels rose by 20–60%.

It is suggested that the increase in mean plasma LH levels in gonadectomized fowl (30–77 ng/ml) over those in cockerels (7–16 ng/ml) is related to an increase in the frequency of the episodic release of the hormone.

Episodic discharges of LH could not be demonstrated in laying hens. In these birds the mean LH levels are low (1·5–2·4 ng/ml) and consequently may be maintained by secretory episodes which are of too low an amplitude to be detectable.

INTRODUCTION

Recent studies in several mammalian species have shown that, over short periods, wide fluctuations in plasma luteinizing hormone (LH) levels within individuals are the result of the hormone being released into the circulation in rapid secretory episodes.

Animals in which this has been demonstrated include castrated and ovariectomized rats (Gay & Sheth, 1972), rams (Katangole, Naftolin & Short, 1974), ovariectomized ewes (Butler, Malven, Willett & Bolt, 1972; Reeves, O'Donnell & Denorscia, 1972; Diekman & Malven, 1973), ovariectomized monkeys (Dierschke, Bhattacharya, Atkinson & Knobil, 1970), intact male monkeys (Mori & Hafez, 1973), intact bulls (Katangole, Naftolin & Short, 1971), men (Dolais, Valleron, Grapin & Rosselin, 1970; Nankin & Troen, 1971; Boyar, Perlow, Hellman, Kapen & Weitzman, 1972; Naftolin,

Yen & Tsai, 1972), women (Dolais *et al.* 1970; Midgley & Jaffe, 1971; Yen, Tsai, Naftolin, Vandenberg & Ajabor, 1972) and agonadal men and women (Root, DeCherney, Russ, Duckett, Garcia & Wallach, 1972; Wallach, DeCherney, Russ, Duckett, Garcia & Root, 1973). This present investigation was undertaken to determine whether LH is secreted in a similar manner in the domestic fowl.

MATERIALS AND METHODS

The experimental fowl were fully grown and consisted of 11 intact cockerels (seven derived from the Thornber 909 strain, four from the Shaver 288 strain), eight castrated Thornber cockerels, eight ovariectomized Thornber hens and six Thornber laying hens. The gonadectomies were carried out 8–10 weeks after hatching. The fowl were housed singly and maintained on a lighting schedule of 16 h light and 8 h darkness (lights on from 05.10 to 21.10 h) with food and water available at all times.

A preliminary study, involving hourly blood sampling from the wing veins of three Shaver cockerels by means of venepuncture, was undertaken to see if levels of plasma LH fluctuated during the day.

Experiments were subsequently designed to follow more closely the changes occurring in levels of LH in the circulation of individual fowl. Serial blood samples were taken through cannulae positioned in the wing veins of unanaesthetized, unrestrained fowl at intervals ranging between 5 and 120 min for periods of between 3 and 12 h. To investigate the consequences of frequent sampling on plasma LH levels, all 11 intact cockerels were sampled at 30, 60 and 120 min intervals for a period of 12 h on successive days. Four of these cockerels were also sampled at 10 min intervals for 3 h. Several days after the end of these experiments, three blood samples were taken at 3 to 4 h intervals by venepuncture from the wing veins of all the intact cockerels.

Blood samples were taken from gonadectomized birds at either 20 to 30 min intervals for 12 h (five females and four males), 10 min intervals for 3 h (two females and three males) or 5 min intervals for 3 h (one female and one male). Blood samples were taken from five laying hens at 10 min intervals for between 2 and 3·5 h; in one of these birds, the descending part of the preovulatory LH peak was included in the sampling period. One other laying hen was sampled at 30 min intervals for 8 h.

In order to determine if insertion of the cannula into the wing vein affected LH secretion, sampling was started in some birds immediately after cannulation and in others an interval of 4–18 h lapsed between cannulation and the start of blood sampling.

To collect a sample, blood was withdrawn through the cannula into a heparinized syringe; the first 0·2 ml was discarded to remove the heparin (50 i.u./ml) injected into the cannula after each sampling, and a further 0·5 ml was taken for assay. The blood was centrifuged at 1800 *g* and the plasma was separated and stored at −20 °C until required. Where the intervals between sampling were greater than 10 min, the red blood corpuscles were periodically reinjected through the cannula, having first been resuspended in an equal volume of 1% saline and then warmed to body temperature. During experiments involving 5- and 10-min sampling, red blood corpuscles were not reinjected, as it was thought that this procedure might have a short-term effect on the release of LH.

Plasma LH levels were measured using an homologous radioimmunoassay for fowl LH (Follett, Scanes & Cunningham, 1972). The standard and ^{125}I-labelled tracer were a preparation of fowl LH (fraction AEI) purified to minimize thyroid-stimulating hormone activity (Scanes & Follett, 1972). Each plasma sample was assayed in duplicate at three dilutions and the statistical analysis carried out using a modified version of a computer program developed by Rodbard & Lewald (1970). Each series of blood samples was assayed in one assay.

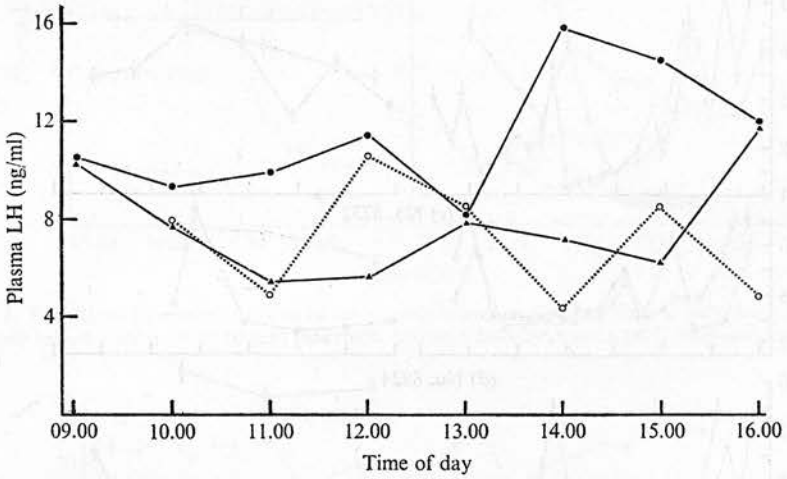

Fig. 1. Variations in concentrations of luteinizing hormone (LH) in plasma samples taken from three intact cockerels (●, ○, ▲) at hourly intervals for 7 h.

RESULTS

Intact cockerels

Plasma LH levels in samples taken hourly from three cockerels by venepuncture fluctuated by as much as 38–52% from individual mean levels of 7·7, 8·2 and 11·5 ng/ml (Fig. 1).

When blood samples were taken from a further 11 birds at 30 min intervals through a cannulated wing vein, two birds showed a rapid drop in plasma LH levels to a steady 2–3 ng/ml whereas the LH levels in the other nine birds, despite an immediate depression, showed fluctuations which took the form of episodic discharges. The LH patterns of four such birds are shown in Fig. 2. Each of these secretory episodes lasted 1·5–2 h and was characterized by a rapid rise and slower fall in plasma LH levels. Ninety-five per cent confidence limits around each plasma LH value distinguished the secretory episodes from some smaller fluctuations that could have been due to intra-assay error.

A more detailed examination of secretory episodes in four cockerels, using 10-min sampling, demonstrated in three of the birds a steep 100–200% increase in plasma LH levels taking place over 10–15 min, followed by a more gradual decline that took the form of an exponential curve (Figs 3*a*, *b* and *c*). The half-life for LH calculated from these slopes was $22{\cdot}9 \pm 5{\cdot}2$ (S.D.) min ($n = 3$). One bird (Fig. 3*d*) failed to show an episodic discharge of LH during the sampling period and it was noticeable that this had the lowest mean plasma LH level of the four birds.

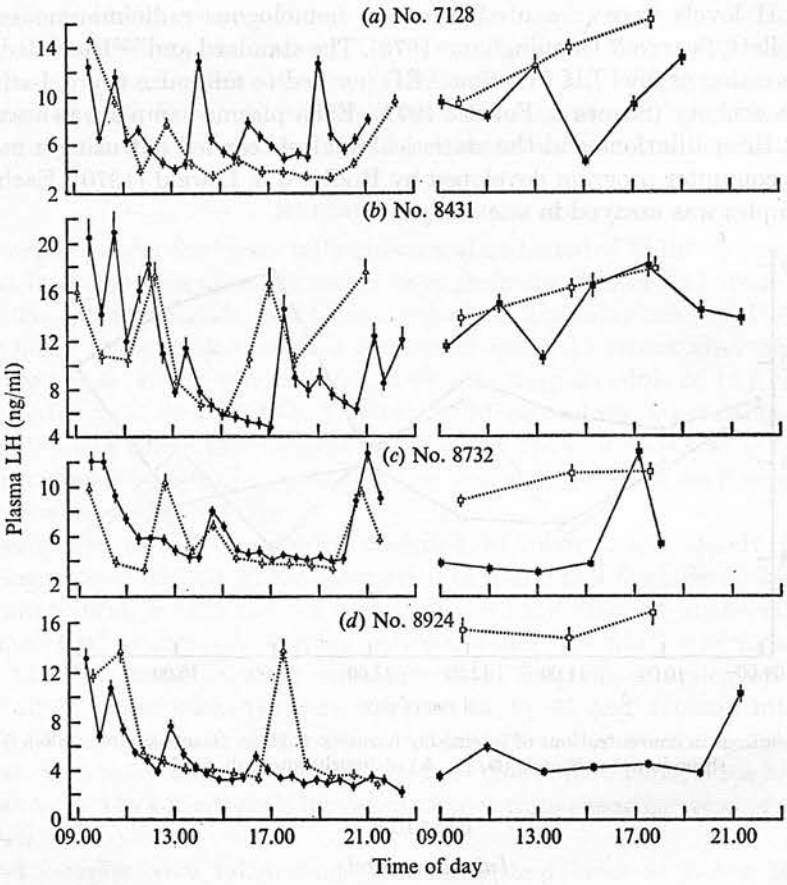

Fig. 2. Variations in concentrations of luteinizing hormone (LH) in plasma samples taken from four intact cockerels on 4 different days at intervals of 30 (●), 60 (△), 120 (■) and 180–240 (□) min. Samples taken from each cockerel were assayed together. Vertical lines represent 95% confidence limits.

The mean plasma LH values obtained for each of the cockerels by averaging the LH concentration in plasma samples taken at 3 to 4 h intervals by venepuncture ranged from 7 to 16 ng/ml. Concentrations of LH in plasma samples taken through a cannula from these same birds at 2 h intervals in some cases (Figs 2*a* and *b*) fell within this range while in others (Figs. 2*c* and *d*) they were depressed below it. Invariably, when blood samples were taken at 60, 30 or 10 min intervals, plasma LH levels fell during the initial sampling period (Figs. 2 and 3*a*, *b* and *d*). This phenomenon was not due entirely to the process of cannulation, since it occurred in birds that had been cannulated 4–18 h before blood samples were taken. The extent to which plasma LH levels were depressed during frequent sampling varied from bird to bird. As plasma LH levels became more depressed, the episodic discharges of the hormone tended to become smaller (Figs 2*d* and 3*c*) and in the birds with the most depressed LH values (2–3 ng/ml) they could not be detected.

No relationship could be established between the pattern of LH secretion and feeding, reinjection of red blood corpuscles or time of day.

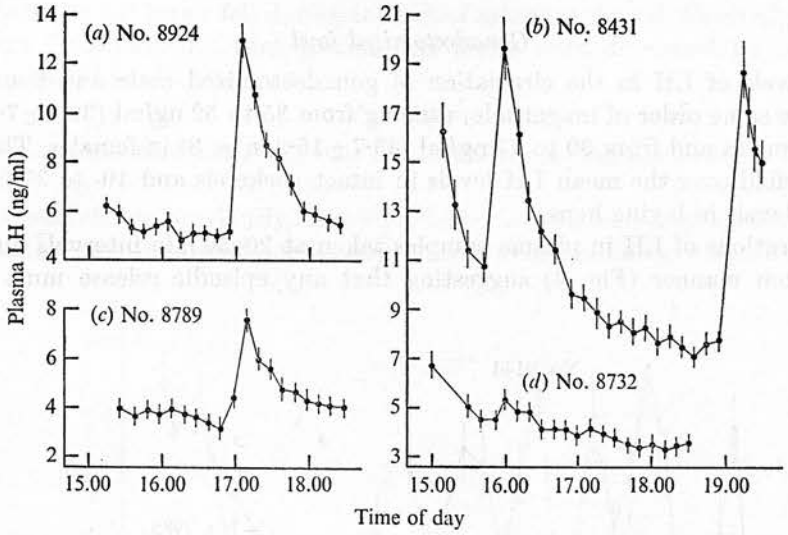

Fig. 3. Variations in concentrations of luteinizing hormone (LH) in plasma samples taken from four intact cockerels at 10 min intervals. Vertical lines represent 95 % confidence limits.

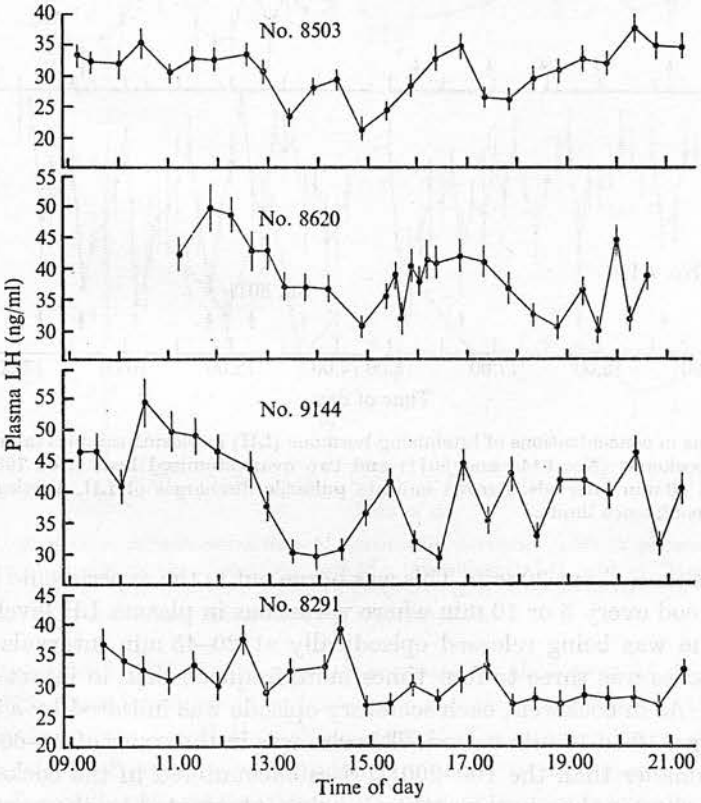

Fig. 4. Variations in concentrations of luteinizing hormone (LH) in plasma samples taken from two ovariectomized hens (Nos 8620 and 8291) and two castrated cockerels (Nos 9144 and 8503) at 20–30 min intervals. Vertical lines represent 95 % confidence limits.

Gonadectomized fowl

Mean levels of LH in the circulation of gonadectomized male and female fowl were of the same order of magnitude, ranging from 35 to 52 ng/ml ($38{\cdot}1 \pm 7{\cdot}9$ (S.D.), $n = 8$) in males and from 30 to 77 ng/ml ($43{\cdot}7 \pm 16{\cdot}2$, $n = 8$) in females. They were raised fourfold over the mean LH levels in intact cockerels and 10- to 30-fold over the mean levels in laying hens.

Concentrations of LH in plasma samples taken at 20–30 min intervals fluctuated in a random manner (Fig. 4) suggesting that any episodic release must have a

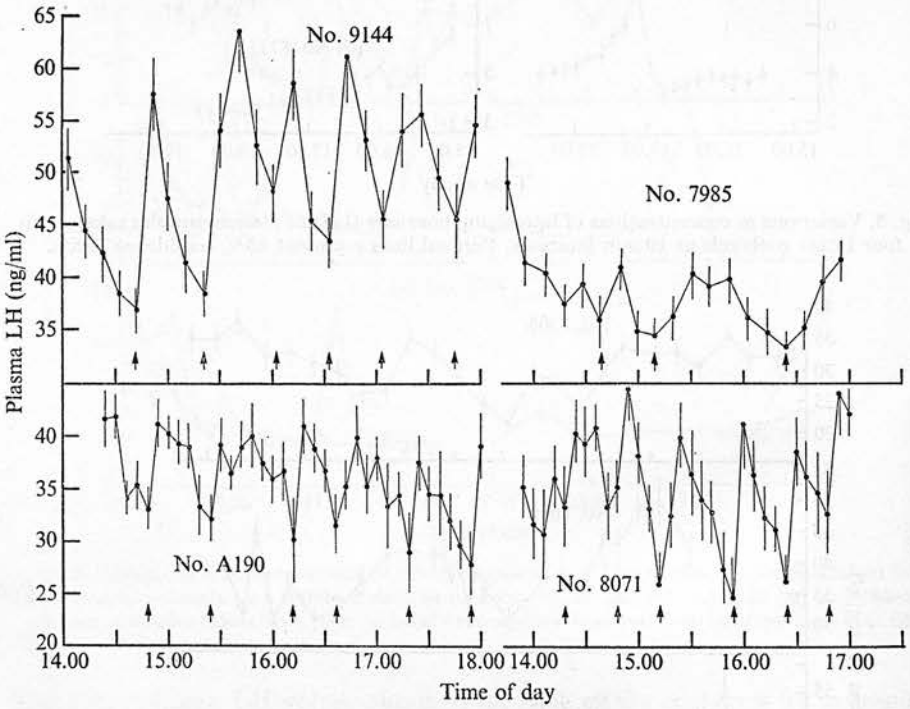

Fig. 5. Variations in concentrations of luteinizing hormone (LH) in plasma samples taken from two castrated cockerels (Nos 9144 and 8071) and two ovariectomized hens (Nos 7985 and A190) at 5 and 10 min intervals. Arrows indicate pulsatile discharges of LH. Vertical lines represent 95% confidence limits.

periodicity of less than about 30 min. This was borne out in the experiments involving withdrawal of blood every 5 or 10 min where variations in plasma LH levels showed that the hormone was being released episodically at 20–45 min intervals (Fig. 5). This episodic release was three to four times more frequent than in intact cockerels (cf Figs 5 and 2). As in cockerels, each secretory episode was initiated by a steep rise in LH levels over a 10 to 15 min period. This rise was in the order of 20–60% which is considerably smaller than the 100–200% rises encountered in the cockerels. The decay phase was shorter than in the cockerels being interrupted by the next episodic discharge. In the case of one bird sampled at 5 min intervals (No. 7985, Fig. 5) an episodic pattern of LH secretion was not clearly seen. This could have been due to

stress since the LH levels fell during the initial sampling period. Generally, however, there was little evidence that plasma LH levels were depressed by handling in gonadectomized fowl.

Intact hens

The mean concentration of plasma LH in the six laying hens ranged from 1·5 to 2·4 ng/ml.

It has been shown previously that when blood is sampled every 1·5 h by means of venepuncture there are no major fluctuations in LH levels during 24 h, other than the

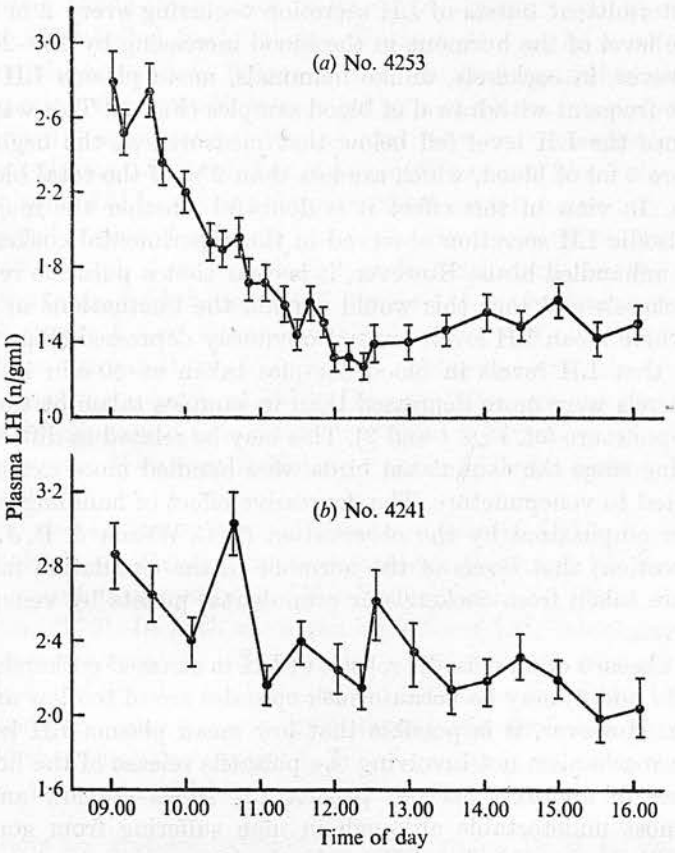

Fig. 6. Variations in concentrations of luteinizing hormone (LH) in plasma samples taken (*a*) from a hen during the descending part of a preovulatory LH peak at 10 min intervals and (*b*) from a hen at 30 min intervals during a portion of the ovulatory cycle when LH levels are at their lowest. Vertical lines represent 95% confidence limits.

preovulatory peak of LH and a small increase at the onset of darkness (Wilson & Sharp, 1973). In this study, when blood was sampled from one hen through a cannula at 30 min intervals, small though significant fluctuations were evident (Fig. 6*b*). However, at this frequency of sampling, no characteristic episodic pattern of secretion was discernible. Sampling from five hens, at 10 min intervals, likewise failed to demonstrate discrete secretory episodes, even when part of the descending slope of the preovulatory LH peak was included in the sampling period (Fig. 6*a*).

DISCUSSION

Previous demonstrations of a pulsatile release of LH have been restricted to mammals (see Introduction for references); its occurrence in the domestic fowl suggests that it reflects a neuroendocrine control mechanism which is common to more than one vertebrate class.

The most striking examples of this mode of secretion in the fowl were seen in cockerels (Figs 2 and 3) where, as in bulls (Katangole *et al.* 1971), men (Nankin & Troen, 1971; Boyar *et al.* 1972; Naftolin *et al.* 1972) and male monkeys (Mori & Hafez, 1973), intermittent bursts of LH secretion occurring every 2 or more hours can result in the level of the hormone in the blood increasing by 100–200% within 10–15 min. However, in cockerels, unlike mammals, mean plasma LH levels were depressed by the frequent withdrawal of blood samples (Fig. 2). This was not due to haemorrhage since the LH level fell below that measured at the beginning of an experiment before 5 ml of blood, which are less than 2% of the total blood volume, were withdrawn. In view of this effect it is doubtful whether the magnitude and frequency of episodic LH secretion observed in the experimental cockerels was the same as that in unhandled birds. However, it is clear that a pulsatile release of LH can occur in cockerels and that this would explain the fluctuations in plasma LH levels in birds where mean LH levels are not obviously depressed (Fig. 1).

It was noted that LH levels in blood samples taken at 60 min intervals from cannulated cockerels were more depressed than in samples taken at the same time interval by venepuncture (cf. Figs 1 and 2). This may be related to differences in the degree of handling since the cannulated birds were handled more extensively than the birds subjected to venepuncture. The depressive effect of handling on LH levels has been further emphasized by the observation (S. C. Wilson & P. J. Sharp, unpublished observation) that levels of the hormone in the circulation fall rapidly if blood samples are taken from cockerels or prepubertal pullets by venepuncture at 10–15 min intervals.

The apparent absence of an episodic release of LH in stressed cockerels and laying hens (Figs. 2*d*, 3*d* and 6) may be because such episodes are of too low an amplitude to be detectable. However, it is possible that low mean plasma LH levels can be maintained by a mechanism not involving the pulsatile release of the hormone. For example, in cases of anorexia nervosa plasma LH levels are low and secretory episodes are almost undetectable although in men suffering from gonadotrophin deficiency and with comparably low LH levels, the hormone is clearly secreted in a pulsatile manner (Santen & Bardin, 1973). In other studies on mammals increased levels of oestrogen in the blood have been shown to depress both the secretion of LH and its episodic release (Yamaji, Dierschke, Bhattacharya & Knobil, 1972; Yen, Tsai, Vandenberg & Rebar, 1972; Diekman & Malven, 1973). The apparent lack of a pulsatile release of LH in the hen may be connected with the way in which ovarian secretions modify the release of LH. It was noticeable that in the one hen in which the descending slope of the preovulatory peak of LH was included in the sampling period no episodic pattern of LH secretion could be detected. This is unlike the situation in women, in which definite episodic releases have been demonstrated during the preovulatory peak of LH (Midgley & Jaffe, 1971; Yen *et al.* 1972). How-

ever, the possibility that episodic discharges of LH could be detected during the rising side of the preovulatory LH peak in the hen remains to be investigated.

As in mammals, removal of the ovaries or testes from the fowl results in increased levels of plasma LH which are maintained by intermittent secretory episodes. The interval between these episodes, 20–45 min, is very similar to that demonstrated in gonadectomized rats (Gay & Sheth, 1972) but is less than in ovariectomized sheep (45–75 min; Dierschke *et al.* 1970) or in a castrated man (approx. 50 min; Santen & Bardin, 1973). The possibility that LH levels are higher in gonadectomized than intact adult fowl because of differences in the biological half-life of the hormone can be discounted. Using the method of Scanes & Follett (1973) it was found that the half-life of ^{125}I-labelled fowl LH was between 17 and 20 min in both intact and gonadectomized birds (S. C. Wilson & P. J. Sharp, unpublished observation).

On comparing the episodic release pattern of LH in intact cockerels and gonadectomized fowl it appears that the interval between the secretory peaks is related to the mean plasma LH level. For example, in cockerel No. 7128 (Fig. 2*a*) in which the mean plasma LH level was 6·9 ng/ml, there were 1–2 secretory episodes every 3 h whereas in the castrated cockerel No. 9144 (Fig. 5), in which the mean plasma LH level was 49·4 ng/ml, there were 4–5 pulsatile discharges over the same period. This suggests that in the fowl the increased plasma LH levels observed after gonadectomy are caused chiefly by an increase in the frequency of the pulsatile release of the hormone from the pituitary. This can be contrasted with the situation in man in which increased mean levels of LH in the circulation are due partly to a percentage increase in the amount of LH released during each secretory episode (Boyar, Perlow, Kapen, Lefkowitz, Weitzman & Hellman, 1973; Naftolin, Judd & Yen, 1973; Santen & Bardin, 1973).

The steep rise and more gradual decline in plasma LH levels characteristic of a secretory episode are identical with the changes in plasma LH levels which follow a single i.v. injection of synthetic LH-releasing hormone (Furr, Onuora, Bonney & Cunningham, 1973). In both cases the half-life of LH, calculated from the rate at which the increased plasma LH levels decline, is about 17–23 min and is the same as the biological half-life of ^{125}I-labelled fowl LH (Scanes & Follett, 1973). This suggests that LH secretion can cease entirely during the declining phase of a secretory episode and that the pulsatile pattern of LH release is a consequence of intermittent and abrupt discharges of LH-releasing hormone.

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